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Drinking Water Criteria Document for 2,3,7,8-Tetrachlorodibenzo-p-dioxin (Final Draft)

(U.S.) Environmental Protection Agency
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DRINKING WATER CRITERIA DOCUMENT FOR 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN

Prepared for

OFFICE OF DRINKING WATER

Prepared by

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The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on 2,3,7,8-TCDD. This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of 2,3,7,8-TCDD
- Toxicokinetics and human exposure to 2,3,7,8-TCDD
- Health Effects of 2,3,7,8-TCDD in humans and animals
- Mechanisms of toxicity of 2,3,7,8-TCDD
- Quantification of toxicological effects of 2,3,7,8-TCDD

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LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
AHH	Aryl hydroxycarbon hydroxylase
bw	Body weight
BCF	Bioconcentration factor
BromoPeCDD	Bromopentachlorodibenzo-*p*-dioxin
DCDD	Dichlorodibenzo-*p*-dioxin
DMBA	Dimethylbenzanthracene
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
EC/GC	Electron capture/gas chromatography
ED_{50}	Median effective dose
FEL	Frank-effect level
GC/MS	Gas chromatography/mass spectrometry
GC/SIM/MS	Gas chromatography/specific ion monitoring/mass spectrometry
GI	Gastrointestinal
HPLC	High performance liquid chromatography
HRGC	High resolution gas chromatography
HRMS	High resolution mass spectrometry
HxCDDs	Hexachloro derivatives of dibenzo-*p*-dioxins
i.p.	Intraperitoneal
LC_{50}	Concentration lethal to 50% of recipients
LD_{50}	Dose lethal to 50% of recipients
LOAEL	Lowest-observed-adverse-effect level
LRMS	Low resolution mass spectrometry
3-MC	3-Methylcholanthrene

MFO	Mixed function oxidase
NICI	Negative ion chemical ionization
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
OCDD	Octachlorinated dibenzo-*p*-dioxins
PCDDs	All polychlorinated dibenzo-*p*-dioxins
PCP	Pentachlorophenol
PeCDDs	Pentachloro derivatives of dibenzo-*p*-dioxins
ppb	Parts per billion
ppm	Parts per million
ppt	Parts per trillion
RBC	Red blood cells
RNA	Ribonucleic acid
SA	Satellite association
s.c.	Subcutaneous
TCDDs	Tetrachloro derivatives of dibenzo-*p*-dioxins
TriCDD	Trichlorodibenzo-*p*-dioxin
2,4,5-T	2,4,5-Trichlorophenoxy acetic acid
TWA	Time-weighted average
UV	Ultraviolet
WCOT	Wall-coated open tubular

I. SUMMARY

2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) is one of the most toxic and environmentally stable tricyclic aromatic compounds belonging to chlorinated dibenzo-p-dioxins. It is a contaminant formed in the production of 2,4,5-trichlorophenol. It is also a contaminant of a few chlorinated phenoxy acids (especially the herbicide 2,4,5-trichlorophenoxy acetic acid and silvex) and hexachlorophene. 2,3,7,8-TCDD is considered relatively stable toward heat, acids and alkalies. It begins to decompose at 500°C and virtually complete decomposition occurs within 21 seconds at a temperature of 800°C. It is very slightly soluble in water (0.2 μg/ℓ). In aquatic media, the compound is expected to persist for a long time since it is likely to remain sorbed to sediments and biota.

2,3,7,8-TCDD is readily absorbed by mammals following either oral or dermal exposure. Because of its relatively high lipid solubility, 2,3,7,8-TCDD is rapidly distributed to tissues with a high lipid content. The liver also represents a major site of accumulation in many species. Metabolism occurs slowly, and the polar metabolites are excreted in the urine and feces. Biliary excretion of 2,3,7,8-TCDD metabolites also occurs. Unmetabolized 2,3,7,8-TCDD is also excreted in the feces and in the milk of lactating animals.

There are great differences in species sensitivity to 2,3,7,8-TCDD, with LD_{50}s ranging from 0.6 μg/kg bw in the guinea pig to 5 mg/kg bw in the hamster. In all species tested, thymic atrophy and severe weight loss are characteristic of 2,3,7,8-TCDD poisoning, with death occurring several days to weeks following exposure. In rats, rabbits and mice, 2,3,7,8-TCDD produces acute liver injury which is not observed in either monkeys, hamsters or guinea pigs. Suppression of the immune system has been observed in mice, rats and guinea pigs. Clinical case studies and epidemiological studies have implicated 2,3,7,8-TCDD as the causative agent in the development of chloracne, hyperpigmentation, altered liver function, porphyria cutanea tarda and hirsutism in humans. In many respects, the type and extent of effects observed are similar to those observed in nonhuman primates, though there are differences in the dermal symptomology elicited. Chloracne is the major dermal finding in humans. In monkeys, common dermal effects include loss of hair, toenails and fingernails.

2,3,7,8-TCDD has been demonstrated to be teratogenic in rats, mice and rabbits, and feticidal in monkeys. The major toxic signs and terata observed were cleft palate in mice, edema, hemorrhage, and kidney anomalies in rats and extra ribs in rabbits. Some epidemiological studies have indicated a possible teratogenic effect in humans, but the evidence is not sufficient to reach a firm conclusion.

In vivo and *in vitro* mutagenicity tests have produced inconclusive evidence as to the mutagenicity of 2,3,7,8-TCDD; however, a number of bioassays have demonstrated this compound to be a potent animal carcinogen. Adenomas or carcinomas of the thyroid, hepatocellular carcinomas, carcinomas of the tongue and hard palate, and adenomas of the adrenal gland have all been produced in rats and mice. Some evidence from human epidemiologic studies associate exposure to herbicides contaminated with 2,3,7,8-TCDD with soft tissue sarcomas and nonHodgkins lymphomas; however, the exposures to 2,3,7,8-TCDD were always compounded with exposures to herbicide chemicals.

These epidemiologic studies are consistent with the position that 2,3,7,8-TCDD is probably carcinogenic for humans. Because 2,3,7,8-TCDD is usually found in association with other materials (e.g., chlorophenols, phenoxyacetic acids, combustion products, etc.), it is not presently possible to evaluate the carcinogenicity of 2,3,7,8-TCDD by itself in humans.

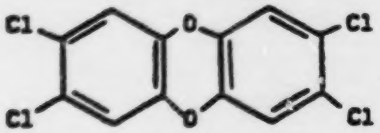
A few possible mechanisms of toxicity have been proposed for 2,3,7,8-TCDD. These include receptor mediated toxicity, metabolism/disposition, vitamin A depletion, increased lipid peroxidation and endocrine imbalance. All of the proposed mechanisms may account for some of the toxic effects of 2,3,7,8-TCDD and/or the species differences in sensitivity to the toxin. However, further research is needed on the mechanism(s) for toxicity of 2,3,7,8-TCDD. Six metabolites have been identified in dogs exposed to 2,3,7,8-TCDD. The major metabolite is 1,3,7,8-tetrachloro-2-hydroxydibenzo-p-dioxin.

A 1-day HA of 3.5×10^{-3} μg/ℓ for a 70 kg adult and 1.0×10^{-3} μg/ℓ for a 10 kg child were calculated from a single-dose oral LOAEL in guinea pigs, the species most sensitive to the toxicity of this compound. Ten-day HAs were calculated by dividing the 1-day HAs by 10^{-4}. The resulting HAs were 3.5×10^{-5} μg/ℓ for a 70 kg adult and 1.0×10^{-4} μg/ℓ for a 10 kg child. A lifetime AADI of 3.5×10^{-5} μg/ℓ was estimated from a LOAEL derived in a 3-generation reproductive study in rats (Murray et al., 1979) along with the rationale developed by the EPA. However, the carcinogenicity risk assessment indicates lower HAs for lifetime exposure to 2,3,7,8-TCDD. Based on a linearized multistage model, and dose-response data for the incidence of tumors of the liver, lung, hard palate or nasal turbinates in female rats, the concentrations of 2,3,7,8- TCDD in drinking water that would result in increased lifetime cancer risks of 10^{-4}, 10^{-5}, and 10^{-6} were estimated to be 2.2×10^{-5}, 2.2×10^{-6} and 2.2×10^{-7} μg/ℓ, respectively.

II. PHYSICAL AND CHEMICAL PROPERTIES

Chemical Structure and Synonyms

2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD)



CAS Registry Number: 1746-01-6

Chem. Abst. Name: 2,3,7,8-tetrachlorodibenzo[b,e](1,4)-dioxin

RTECS Number: HP35000

Synonyms: Dioxin; TCDBD; TCDD; 2,3,7,8-tetrachlorodibenzodioxin, 2,3,7,8-tetrachlorodibenzo-1,4-dioxin (IARC, 1977).

Physical Properties

2,3,7,8-TCDD has a molecular formula of $C_{12}H_4Cl_4O_2$ and a molecular weight of 321.9. In the pure form, it exists as colorless needles with a melting point of 303-305°C (Crummett and Stehl, 1973). In a chloroform solution, the maximum absorption of 2,3,7,8-TCDD occurs at 310 nm, with a molar absorption coefficient of 5562 (NRCC, 1981). The available solubility data (Table II-1) indicates that 2,3,7,8-TCDD is a highly lipophilic substance.

Values for other physical properties of 2,3,7,8-TCDD that are available in the literature searched are given in Table II-2.

TABLE II-1

Solubility of 2,3,7,8-TCDD*

Solvent	Solubility (ppm)
Water	2×10^{-4}
Lard oil	44
Benzene	570
o-Dichlorobenzene	1400
Chloroform	370
Acetone	110
n-Octanol	50
Methanol	10

*Source: Adapted from Crummet and Stehl, 1973

TABLE II-2

Physical Parameters of 2,3,7,8-TCDD

Parameter	Value	Reference
Vapor pressure (mm of Hg)	1.7×10^{-6}	NRCC, 1981
Octanol/water partition coefficient	1.4×10^{6} 6.9×10^{6} 1.9×10^{7}	NRCC, 1981 Mabey et al., 1981 U.S. EPA, 1984a
Sorption partition coefficient (K_{OC})	9.9×10^{5} 3.3×10^{6}	NRCC, 1981 Mabey et al., 1981

Stability

2,3,7,8-TCDD is considered relatively stable toward heat, acids and alkalies (Albro, 1979). It begins to decompose at a temperature of 500°C with virtually complete degradation at 800°C within 21 seconds (Stehl et al., 1973). Gamma radiation degrades 2,3,7,8-TCDD (Fanelli et al., 1978).

The four transformation processes (photoreaction, biotransformation, hydrolysis and radical oxidation) that control the fate of a chemical in aquatic media do not appreciably transform 2,3,7,8-TCDD (Matsumura et al., 1983). In organic solvents, 2,3,7,8-TCDD undergoes reductive photodechlorination at wavelengths <320 nm (Crosby et al., 1971; Liberti et al., 1978). In aqueous solution, hydroxylative dechlorination has not been seen. Plimmer et al. (1973) reported that a 2,3,7,8-TCDD suspension in distilled water remained unchanged when irradiated with a sunlamp for an unspecified time. In contrast, 2,3,7,8-TCDD in methanol solution, or a benzene solution of 2,3,7,8-TCDD in water in the presence of a surfactant, underwent substantial photodegradation under sunlamp or sunlight irradiation (Plimmer et al., 1973; Crosby et al., 1971). The surfactant, 1-hexyldecylpyridinium chloride, sensitized the photodecomposition of 2,3,7,8-TCDD in aqueous solution (Botre et al., 1978). In order to explain the longer half-life of 2,3,7,8-TCDD (1.6 year vs. 1 year) in a model laboratory ecosystem than in an outdoor pond, Matsumura et al. (1983) and Tsushimoto et al. (1982) speculated that photolysis is the most likely cause. In the outdoor environment, where the intensity of sunlight is higher compared with the laboratory experiments (100 w fluorescent lamp over 40 cm of water surface), algae-mediated photosensitization of 2,3,7,8-TCDD was speculated to have caused some photodecomposition of this compound. From the available information, it is difficult to predict the fate of 2,3,7,8-TCDD in aquatic media under environmental photolytic conditions. In the presence of hydrogen atom donating substrate(s) in surface waters, photolysis may be a significant fate process.

2,3,7,8-TCDD exhibits relatively strong resistance to microbial biodegradation. Only 5 of ~100 microbial strains that have the ability to degrade persistent pesticides show slight ability to degrade 2,3,7,8-TCDD (U.S. EPA, 1980). Ward and Matsumura (1977) reported that the half-life of 2,3,7,8-TCDD in sediment-containing Wisconsin lake waters was 550-590 days. In lake water alone, ~70% of the 2,3,7,8-TCDD remained after 589 days. Using an outdoor pond as a model aquatic ecosystem, Tsushimoto et al. (1982) and Matsumura et al. (1983) estimated the apparent half-life of 2,3,7,8-TCDD to be ~1 year. Although biodegradation may have been responsible for part of the degradation, other investigators (Huetter and Philippi, 1982) have reported the virtually complete lack of biodegradability of 2,3,7,8-TCDD.

The biodegradation half-life of 2,3,7,8-TCDD can be estimated from the theoretical rate constant values based on relative rates of transformation reported in the literature or on structure-activity analogy values given by Mabey et al. (1981). Assuming the biotransformation rate constant of $1\text{x}10^{-10}$ mℓ cell^{-1} hr^{-1} (Mabey et al., 1981) and the concentration of microorganisms capable of degrading 2,3,7,8-TCDD as $5\text{x}10^{3}$ cell $\text{m}\ell^{-1}$ (Burns et al., 1981), the half-life of biodegradation is estimated to be >1 year.

Although several investigators implicated volatilization as one of the major reasons for the observed disappearance of 2,3,7,8-TCDD from aqueous solution during microbial studies, little quantitative information regarding the volatilization of 2,3,7,8-TCDD from aquatic media is available. 2,3,7,8-TCDD may undergo some water-mediated evaporation in aquatic media (Matsumura et al., 1983). A transport model to estimate 2,3,7,8-TCDD volatilization from a cooling pond on an industrial site based on measured concentrations in the pond bottom sediment and pond surface area led to an estimated rate of 15-16 mg/year (Thibodeaux, 1983). Using the formulas of Liss and Slater (1974), a vapor pressure value of -10^{-6} torr (0.1 m Pa) and a solubility value of 6.2×10^{-10} mole/ℓ, NRCC (1981) calculated the volatilization half-life for 2,3,7,8-TCDD to be 6 minutes from water of 1 cm depth and 10 hours from water of 1 m depth. Evaporation half-life is directly proportional to water depth and inversely proportional to mass transfer coefficient (Thibodeaux, 1979). The limitations of the Liss-Slater theory to predict the rate of volatilization have been discussed in the NRCC (1981) document. The Liss-Slater model does not consider terrestrial matrices (suspended solids, sediments, biota, etc.) normally encountered in natural surface water. Employing a computerized EXAMS model for two standardized aquatic ecosystems and the input parameters for 2,3,6,8-TCDD, as discussed in NRCC (1981), volatilization has been estimated to account for 100% of the fraction lost and biodegradation has been calculated to be 0%. The volatilization half-life for 2,3,7,8-TCDD has been estimated to be 5.5 and 12 years from pond and lake water, respectively. However, it should be remembered that these are estimated values and experimental confirmation of these values is not available.

Data from microcosm experiments indicate that 2,3,7,8-TCDD is highly sorbed to sediments and biota (Isensee and Jones, 1975; Ward and Matsumura, 1978). More than 90% of 2,3,7,8-TCDD in an aquatic medium may be present in the adsorbed state (Ward and Matsumura, 1978; Matsumura et al., 1983). This is not surprising considering the low water solubility and the high octanol/water partition coefficient. In fact, the equation of Karickhoff et al. (1979) predicts a sorption partition coefficient value of 10^4 for 2,3,7,8-TCDD in sediments containing 2% organic carbon (NRCC, 1981).

Summary

2,3,7,8-TCDD has a molecular formula of $C_{12}H_4Cl_4O_2$ and a molecular weight of 321.9. In pure form, it exists as colorless needles with a melting point of 303-305°C (Crummett and Stehl, 1973) and is relatively resistent to degradation by heat, acids and alkalies. It is very slightly soluble in water (0.2 ppb) and somewhat soluble in organic solvents (Crummett and Stehl, 1973). The compound has a low vapor pressure (1.7×10^{-6} mm of Hg) and a high octanol/water partition coefficient (NRCC, 1981; Mabey et al., 1981).

From the available information, it is difficult to predict the photolytic fate of 2,3,7,8-TCDD in aquatic media. In the absence of hydrogen donating substrates, photolysis does not appear to be a significant process (Plimmer et al., 1973; Crosby et al., 1971). 2,3,7,8-TCDD exhibits relatively strong resistance to microbial degradation (U.S. EPA, 1980; Huetter and Philippi, 1982). The esimated biodegradation half-life of 2,3,7,8-TCDD is >1 year (Mabey et al., 1981; Burns et al., 1981). Hydrolysis and radical oxidation do not appear to be significant processes for 2,3,7,8-TCDD in aquatic media (NRCC, 1981).

Little quantitative information regarding the volatilization of 2,3,7,8-TCDD from aquatic media is available. Theoretical modeling of 2,3,7,8-TCDD (EXAMS model) provides a volatilization half-life for 2,3,7,8-TCDD of 5.5 and 12 years from pond and lake water, respectively (NRCC, 1981). However, no experimental confirmation of these values is available. Experimental data show that this compound is likely to remain sorbed to sediments and biota in aquatic media (Isensee and Jones, 1975; Ward and Matsumura, 1978).

III. TOXICOKINETICS

The toxicokinetics of 2,3,7,8-TCDD has been investigated in a number of laboratory animals, and there are several recent reviews on this subject (Neal et al., 1982; Gasiewicz et al., 1983a; Olson et al., 1983). This section will examine our current understanding of the absorption, distribution, metabolism and excretion of 2,3,7,8-TCDD in various mammalian species.

Absorption

The dermal and gastrointestinal absorption of 2,3,7,8-TCDD have been investigated in several species. No studies are available on the toxicokinetics of 2,3,7,8-TCDD through the inhalation route of exposure.

Absorption From the Gastrointestinal Tract. Experimentally, 2,3,7,8-TCDD is generally administered in the diet or by gavage in an oil vehicle. In Sprague-Dawley rats given a single oral dose of 1.0 μg [^{14}C]2,3,7,8-TCDD/kg bw, absorption from the intestinal tract was estimated at ~83% (Rose et al., 1976). With repeated oral dosing at 1.0 μg/kg/day (5 days/week x 7 weeks), absorption was observed to be approximately that observed for the single oral dose. With a much larger single oral dose, 50 μg/kg bw, ~70% of the dose was absorbed by Sprague-Dawley rats (Piper et al., 1973). In these studies, the chemical was administered by gavage in acetone:corn oil (1:25 or 1:9). One study in the guinea pig reported that ~50% of a single oral dose (quantity not mentioned) of 2,3,7,8-TCDD in acetone:corn oil was absorbed (Nolan et al., 1979). The gastrointestinal absorption of 2,3,7,8-TCDD was also examined in the hamster, the species most resistant to the acute toxicity of this toxin (Olson et al., 1980a). Olson et al. (1980a) administered hamsters a single, sublethal, oral dose of

[1,6-^{3}H]-2,3,7,8-TCDD in olive oil (650 μg/kg) and reported that 74% of the dose was absorbed. When 2,3,7,8-TCDD was administered to rats in the diet at 7 or 20 ppb (0.5 or 1.4 μg/kg/day) for 42 days, 50-60% of the consumed dose was absorbed (Fries and Marrow, 1975). These findings indicate that over a wide range of doses and under these experimental conditions, 2,3,7,8-TCDD is generally well absorbed from the gastrointestinal tract of the three species that have been examined.

Contact with 2,3,7,8-TCDD in the environment would most often involve exposure to a complex mixture containing the toxin, as opposed to the above experimental situation, where 2,3,7,8-TCDD was administered in the diet or through an oil vehicle.

The influence of dose and vehicle or adsorbent on gastrointestinal absorption has been investigated in rats by Poiger and Schlatter (1980), using hepatic concentrations 24 hours after dosing as an indicator of the amount absorbed. They found a linear relationship between ng 2,3,7,8-TCDD administered in 50% ethanol (for doses of 12-280 ng, equivalent to 0.06-1.4 μg/kg) and the percentage of the dose in hepatic tissues (36.7-51.5%). At the next higher dose of 1070 ng, however, the percentage fell off to about 42%. Their results regarding the influence of vehicle or adsorbent on gastrointestinal absorption have been summarized in Table III-1. Administration of 2,3,7,8-TCDD in an aqueous suspension of soil resulted in a decrease in the hepatic levels of 2,3,7,8-TCDD as compared with hepatic levels resulting from administration of 2,3,7,8-TCDD in 50% ethanol. The extent of the decrease was directly proportional to the length of time the 2,3,7,8-TCDD had been in contact with the soil. When 2,3,7,8-TCDD was mixed

TABLE III-1

Percentage of 2,3,7,8-TCDD in the Liver of Rats 24 Hours After Oral Administration of 0.5 mℓ of Various Formulations Containing TCDD*

Formulation	TCDD Dose (ng)	No. of Animals	Percentage of Dose in the Liver
50% Ethanol	14.7	7	36.7 ± 1.2
Aqueous suspension of soil (37%, w/w) that had been in contact with TCDD for:			
10-15 hours	12.7, 22.9	17	24.1 ± 4.8
8 days	21.2, 22.7	10	16.0 ± 2.2
Aqueous suspension of activated carbon (25%, w/w)	14.7	6	≤0.07

*Source: Adapted from Poiger and Schlatter, 1980 in an aqueous suspension of activated carbon, absorption was almost totally eliminated (<0.07% of the dose in hepatic tissues).

Philippi et al. (1981) and Hutter and Philippi (1982) have shown that radiolabeled 2,3,7,8-TCDD becomes progressively more resistant with time to extraction from soil. Similarly, the feeding of fly ash, which contains PCDDs, to rats in the diet for 19 days resulted in considerably lower hepatic levels of PCDDs than did the feeding of an extract of the fly ash at comparable dietary concentrations of PCDDs (van den Berg et al., 1983). The PCDDs were tentatively identified as 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,6,7,8-HxCDD and 1,2,3,7,8,9-HxCDD and the difference in hepatic levels noted between fly ash-treated and extract-treated rats was greater for the more highly chlorinated isomers than it was for 2,3,7,8-TCDD. These results indicate the importance of the formulation or vehicle containing the toxin(s) on the relative bioavailability of 2,3,7,8-TCDD, PeCDD and HxCDDs following oral exposure.

Information on the absorption of 2,3,7,8-TCDD through the skin is found only in a study by Poiger and Schlatter (1980). The authors administered 26 ng 2,3,7,8-TCDD in 50 μℓ methanol to the skin of six rats. After 24 hours, the liver contained 14.8±2.6% of the dose. By comparing to the hepatic levels obtained after oral administration in 50% ethanol (in the same study), the amount absorbed from a dermal application can be estimated at ~40% of the amount absorbed from an equivalent oral dose. This comparison assumes that hepatic levels are valid estimates of the amount absorbed from both oral and dermal routes and that absorption from methanol is equivalent to absorption from 50% ethanol. As compared with dermal application in methanol, dermal application of 2,3,7,8-TCDD to rats in vaseline or polyethylene glycol reduced the percentage of the dose in hepatic tissue to 1.4 and 9.3%, respectively, but had no observable effect on the dose of 2,3,7,8-TCDD required to induce skin lesions (~1 µg/ear) in the rabbit ear assay. Application of 2,3,7,8-TCDD in a soil/water paste decreased hepatic 2,3,7,8-TCDD to ~2% of the administered dose and increased the amount required to produce skin lesions to 2-3 µg in rats and rabbits, respectively. Application in an activated carbon/water paste essentially eliminated absorption, as measured by percent of dose in the liver, and increased the amount of 2,3,7,8-TCDD required to produce skin lesions to ~160 µg. These results suggest that the dermal absorption and acnegenic potency of 2,3,7,8-TCDD are dependent on the formulation (vehicle or adsorbent) containing the toxin.

Distribution

The tissue distribution of 2,3,7,8-TCDD in a number of species is summarized in Table III-2. From these data it is apparent that 2,3,7,8-TCDD distributes preferentially to the liver and adipose tissue of most species that have been examined. Piper et al. (1973) used a single oral dose of $[^{14}C]$2,3,7,8-TCDD to study distribution and excretion in male Sprague-Dawley rats. Most of the radioactivity (53.2%) was excreted via the feces, but the urine and expired air accounted for 13.2 and 3.2%, respectively. Analysis of the tissues after 3 days showed liver and adipose tissue to contain the highest percent of the dose per gram of tissue, with 3.18 and 2.60%, respectively.

TABLE III-2

Tissue Distribution of 2,3,7,8-TCDD

Species	Route of Administration	Tissues with the Highest Concentration of 2,3,7,8-TCDD	References
Rat	oral	liver	Fries and Marrow, 1975
Rat	oral	liver > fat	Rose et al., 1976
Rat	oral	liver > fat	Piper et al., 1973
Rat	oral	liver > fat	Kociba et al., 1978a,b
Rat	oral	liver > fat	Allen et al., 1975
Rat	i.p.	liver > fat	Van Miller et al., 1976
Mouse	oral	liver > fat > kidney > lung	Manara et al., 1982
Mouse	i.p.	liver > fat > kidney > lung > spleen	Manara et al., 1982
Rhesus monkey	i.p.	fat > skin > liver > adrenal = thymus	Van Miller et al., 1976
Golden Syrian hamster	i.p. or oral	liver > fat	Olson et al., 1980a
Guinea pig	oral	fat > liver > adrenals > thymus > skin	Nolan et al., 1979
Guinea pig	i.p.	fat > liver > skin > adrenals	Gasiewicz and Neal, 1979

Rose et al. (1976) also examined the distribution of [^{14}C]2,3,7,8-TCDD in the rat. Twenty-two days after a single oral dose of 1.0 µg/kg, liver and adipose tissue had retained most of the ^{14}C activity, with 1.26 and 1.25% of the label retained per gram of tissue, respectively. With repeated oral doses, the activity was again localized mainly in the liver and adipose tissue, but the liver had five times as much radioactivity as did the fat. With the single oral dose, no radioactivity was detected in either the urine or expired air, indicating that most if not all of the elimination of 2,3,7,8-TCDD and/or its metabolites was through the feces. With repeated oral doses, the ^{14}C activity was also excreted primarily through the feces, but significant amounts were found in the urine, especially of the female rats. Male rats given 1.0 µg/kg/day of 2,3,7,8-TCDD for 7 weeks excreted an average of 3.1% of the cumulative dose in the urine while the female rats excreted an average of 12.5% in the urine (Rose et al., 1976). Fries and Marrow (1975) have also reported evidence of sex differences in tissue distribution in rats. During 42 days of administration of 2,3,7,8-TCDD, ~85% of the total body residue of male rats was located in the liver, while 70% of the total body residue of female rats was located in this organ.

Studies performed by Van Miller et al. (1976) on rhesus monkeys and rats using single i.p. doses of tritiated 2,3,7,8-TCDD (400 µg/kg bw) showed that while rats had over 40% of the 2,3,7,8-TCDD in the liver 7 days after dosing, the monkeys had only about 10% in the same organ at that time. In two strains of mice, the liver contained ~35% of an administered dose of 2,3,7,8-TCDD 1 day after oral or i.p. administration (Manara et al., 1982). The liver was also found to be the major site of accumulation of 2,3,7,8-TCDD in the hamster, with 20% of the dose localized in the liver (5.3% of dose/g liver) at 3 days following a sublethal dose of 650 μg ^{3}H-2,3,7,8-TCDD/kg (Olson et al., 1980a). In all three species, 1-22 days after single-dose oral or i.p. administration, levels of 2,3,7,8-TCDD in adipose tissue were generally slightly lower than levels in the liver, and were considerably higher than concentrations in other tissues (Piper et al., 1973; Rose et al., 1976; Van Miller et al., 1976; Olson et al., 1980a; Manara et al., 1982), including the thymus (Rose et al., 1976; Van Miller et al., 1976; Olson et al., 1980a).

Kociba et al. (1978a,b) found that female rats maintained on a daily dietary 2,3,7,8-TCDD intake of 0.1 μg/kg/day for 2 years had an average 2,3,7,8-TCDD content of 8100 ppt in fat and 24,000 ppt in the liver. Rats given 0.01 μg/kg/day had an average of 1700 ppt of 2,3,7,8-TCDD in the fat and 5100 ppt in the liver. For both of these daily dosages the liver:body fat ratio of 2,3,7,8-TCDD was 3:1. At the lowest dose level of 0.001 μg/kg/day, both fat and liver contained an average of 540 ppt 2,3,7,8-TCDD. Kociba et al. (1976) presented evidence that steady-state had been reached after <13 weeks of feeding of 2,3,7,8-TCDD.

McNulty et al. (1982) reported that 2 years after administration of a single oral dose of 1 μg/kg of 2,3,7,8-TCDD to an adult rhesus macaque monkey, tissue levels of the compound were 100 ppt in adipose tissue and 15 ppt in liver. These results indicate that prolonged retention of 2,3,7,8-TCDD may occur in this species. The tissue distribution of 2,3,7,8-TCDD in the guinea pig appears to be similar to the monkey, with the highest concentration of the toxin being found in adipose tissue (Gasiewicz and Neal, 1979; Nolan et al., 1979). The interspecies difference in the tissue distribution of 2,3,7,8-TCDD may be related to the relative adipose tissue content of a given species and/or the affinity of 2,3,7,8-TCDD for the hepatic microsomal fraction; however, the significance of these differences remains in doubt. For example, the hepatotoxicity of 2,3,7,8-TCDD in a given species does not appear to be related to the hepatic concentration of the toxin (Neal et al., 1982).

2,3,7,8-TCDD has been demonstrated to be teratogenic and fetotoxic in the rat (see Teratogenicity section); the ability of 2,3,7,8-TCDD to gain access to the developing fetus of Fischer 344 rats following a single oral dose of [^{14}C]2,3,7,8-TCDD was investigated by Moore et al. (1976). They found low concentrations of 2,3,7,8-TCDD in the fetus at gestation days 14, 18 and 21. The radioactivity appeared to be evenly distributed throughout the fetus on days 14 and 18; however, increased levels of radioactivity were detected in fetal liver on day 21. Nau and Bass (1981) (more recently reported by Nau et al., 1982) investigated the fetal uptake of 2,3,7,8-TCDD in NMRI mice following oral, i.p. or s.c. administration of the compound at dose levels of 5, 12.5 or 25 μg/kg in DMSO:corn oil or acetone:corn oil. The chemical was usually administered as a single dose 2 days prior to sacrifice. All three modes of administration produced similar maternal and embryonic or fetal levels of 2,3,7,8-TCDD at 5 and 12.5 μg/kg. At 25 μg/kg, higher maternal and fetal tissue levels were obtained with s.c. administration, and much higher levels were obtained with i.p. administration, than were obtained with oral administration. Embryonic 2,3,7,8-TCDD concentrations were maximal on gestational days 9 and 10; however, low levels were found in the embryo and fetus between gestational days 11 and 18. This sharp decrease in 2,3,7,8-TCDD concentration coincides with placentation. 2,3,7,8-TCDD concentrations in the placenta were an order of magnitude greater than in the fetus itself. The affinity of fetal liver for 2,3,7,8-TCDD was relatively low, as compared to maternal liver; however, 2,3,7,8-TCDD levels in fetal livers were 2-4 times higher than the levels in other fetal organs. An attempt was made to correlate 2,3,7,8-TCDD levels in the fetuses with the observed incidence of cleft palate, but no clear relationship was observed.

Autoradiographic studies of tissue localization following i.v. administration of [^{14}C]2,3,7,8-TCDD in DMSO to three strains of mice indicated that the liver had the highest concentration and longest retention of radioactivity in the body, followed by the nasal mucosa (Appelgren et al., 1983). In pregnant mice, the concentration of radioactivity in the fetuses was lower than in the dams, but a similar, selective labelling of the liver and the nasal mucosa was seen in the fetuses at day 17 of gestation. In the adult animals, labelling of the adrenal cortex was about equal to that of the liver at 1 hour after dosing, but thereafter was much lower than in the liver. Labelling of the thymus, lymph nodes, bone marrow and prostate were low at all observation times (i.e., 5 minutes to 61 days after injection).

Very few data are available on the tissue distribution of 2,3,7,8-TCDD in humans. Facchetti et al. (1980) reported tissue concentration of 2,3,7,8-TCDD at levels of 1-2 ng/g in liver and $\leq$0.1 ng/g in thyroid, brain, lung, kidney and blood in a woman who died 7 months after potential exposure to 2,3,7,8-TCDD from the Seveso accident. This pattern of 2,3,7,8-TCDD distribution, however, may not be representative for humans since the woman at the time of death had an adenocarcinoma (which was not considered related to the accident) involving the pancreas, liver and lung.

In addition Young et al. (1983) reported preliminary results of the analysis of adipose tissue from soldiers exposed to Agent Orange. Two analyses were performed, one using the exact mass of 321.8936 and the other the signal profile at masses 321.8936 and 319.8965. Three groups were studied consisting of 20 veterans claiming health problems related to Agent Orange exposure, 3 Air Force officers with known heavy exposure to Agent Orange during disposal operations, and 10 control veterans with no known herbicide exposure. In the first group, 10 of the 20 had measurable levels of 2,3,7,8-TCDD (5 with 5-7 ppt, 3 with 9-13 ppt and 1 with 23 and 35 ppt and another with 63 and 99 ppt). In the second group only two officers had measurable 2,3,7,8-TCDD levels and these did not exceed 3 ppt. In the 10 control veterans, 4 had 2,3,7,8-TCDD levels between 7 and 14 ppt. Levels of 2,3,7,8-TCDD in adipose tissue did not appear to be associated in this study with ill health or any particular symptom. However, it was considered that information on background levels of 2,3,7,8-TCDD in adipose tissue was too limited to draw any firm conclusions.

Metabolism

Vinopal and Casida (1973) found no evidence of water soluble metabolites of 2,3,7,8-TCDD following incubation with mammalian liver microsomes or i.p. injection into mice. In the same experiment, only unmetabolized 2,3,7,8-TCDD was extractable from mouse liver 11-20 days after treatment. Van Miller et al. (1976) claimed that the slow elimination of 2,3,7,8-TCDD they observed in both rats and monkeys after i.p. injections suggested that

2,3,7,8-TCDD was not readily metabolized. Metabolites of 2,3,7,8-TCDD have been detected in the bile and urine of Syrian Golden hamsters after single oral or i.p. doses (Olson et al., 1980a) and in the bile of dogs following repeated direct introduction of the chemical into the duodenal lumen (Poiger et al., 1982a).

Poiger and Schlatter (1979), Ramsey et al. (1979) and Ramsey et al. (1982) demonstrated biliary excretion of several metabolites of [^{14}C]2,3,7,8-TCDD by rats after repeated oral dosing. The metabolites were tentatively identified as glucuronides of hydroxylated 2,3,7,8-TCDD. The amounts of metabolites found were small, indicating that 2,3,7,8-TCDD is only slowly metabolized in the liver. Previous work by Piper et al. (1973) using single oral doses of 2,3,7,8-TCDD concluded that, since small amounts of radioactivity were found in the urine and expired air of male rats during the first 10 days, metabolic alteration or breakdown must occur. The study by Rose et al. (1976) using oral doses stated that while the ^{14}C activity in the rat livers appeared to be present as unchanged 2,3,7,8-TCDD, a significant amount of radioactivity found in the feces appeared to come from substances other than 2,3,7,8-TCDD; the excretion of ^{14}C in the urine also indicated that metabolism had occurred.

Poiger et al. (1982a) investigated the toxicity of 2,3,7,8-TCDD metabolites by administering extracts of bile from 2,3,7,8-TCDD-treated dogs to male guinea pigs in single oral doses equivalent to 0.6, 6.0 and 60 μg/kg of parent compound. Other groups of guinea pigs received bile extract from untreated dogs or 2,3,7,8-TCDD itself. A comparison of the mortality data at 5 weeks after dosing indicated that the acute toxicity of 2,3,7,8-TCDD to guinea pigs was at least 100 times higher than was the acute toxicity of its metabolites.

More recently, Olson et al. (1983) reported that all of the radioactivity in urine and bile from ^{14}C-2,3,7,8-TCDD-treated rats, hamsters and guinea pigs corresponded to metabolites of 2,3,7,8-TCDD. The enzymatic hydrolysis of the 2,3,7,8-TCDD metabolites from the rat and hamster altered the chromatographic profile of the metabolites, indicating the presence of glucuronide conjugates in bile and sulfate conjugates in urine (Olson and Bittner, 1983). The apparent absence of these metabolites in extracts of hamster and rat liver suggest that once formed, the metabolites of 2,3,7,8-TCDD are readily excreted (Olson et al., 1980a; Rose et al., 1976). These results also indicate that urinary and biliary elimination of 2,3,7,8-TCDD is dependent upon metabolism of the toxin. Although urine and bile appear to be free of unmetabolized TCDD, data from the hamster and rat indicate that from 10 to 40% of the 2,3,7,8-TCDD-derived radioactivity in feces represents unchanged 2,3,7,8-TCDD (Olson et al., 1983; Olson and Bittner, 1983). The daily presence of unchanged 2,3,7,8-TCDD in feces and its absence in bile suggests that direct intestinal elimination may be the source for the fecal excretion of 2,3,7,8-TCDD. This finding demonstrates that the half-life for elimination of 2,3,7,8-TCDD may not directly reflect the *in vivo* rate of 2,3,7,8-TCDD metabolism in a given animal. Nevertheless, the metabolism of 2,3,7,8-TCDD does in part regulate its elimination or relative persistence in a given animal.

Several metabolites of 2,3,7,8-TCDD have recently been identified. Sawahata et al. (1982) investigated the *in vitro* metabolism of 2,3,7,8-TCDD in isolated rat hepatocytes. The major product was deconjugated with β-glucuronidase, derivatized with diazomethane, and separated into two compounds by high performance liquid chromatography (HPLC). These metabolites were subsequently identified as 1-hydroxy-2,3,7,8-TCDD and 8-hydroxy-2,3,7-trichlorodibenzo-p-dioxin. Poiger et al. (1982a) identified six metabolites in the bile of dogs that were given a lethal dose of [3H]2,3,7,8-TCDD. The major metabolite was 1,3,7,8-tetrachloro-2-hydroxydibenzo-p-dioxin; 3,7,8-trichloro-3-hydroxydibenzo-p-dioxin and 1,2-dichloro-4,5-hydroxybenzene were also identified as minor metabolites. The structures of the three remaining metabolites were not determined; however, two appeared to be trichlorohydroxydibenzo-p-dioxins and the third was apparently a chlorinated 2-hydroxydiphenyl ether.

Data on the metabolism of 2,3,7,8-TCDD suggests that reactive epoxide intermediates may be formed. Poland and Glover (1979) have investigated the *in vivo* binding of [1,6-^{3}H]-2,3,7,8-TCDD derived radioactivity to rat hepatic macromolecules. They found maximum levels equivalent to 60 pmol 2,3,7,8-TCDD/mole of amino acids in protein, 12 pmol 2,3,7,8-TCDD/mole of nucleotide in rRNA, and 6 pmol of 2,3,7,8-TCDD/mole of nucleotide in DNA. This corresponds to one 2,3,7,8-TCDD-DNA adduct/35 cells. Poland and Glover (1979) suggest that it is unlikely that 2,3,7,8-TCDD-induced oncogenesis is through a mechanism of covalent binding to DNA and somatic mutation. Further studies in other species, possibly with [^{14}C]-2,3,7,8-TCDD, are needed to confirm these results and assess the relationship between covalent binding and the short and long-term toxicity of 2,3,7,8-TCDD.

Isolated rat hepatocytes in suspension have been used as an in vitro system for assessing 2,3,7,8-TCDD metabolism under various conditions (Olson et al., 1981). Data indicate that the rate of 2,3,7,8-TCDD metabolism in rat hepatocytes correlates directly with drug induced changes in hepatic cytochrome P-450 monooxygenase activity, suggesting that 2,3,7,8-TCDD is metabolized by this enzyme (Neal et al., 1982). Pretreatment of rats with 2,3,7,8-TCDD has been shown to enhance the rate of 2,3,7,8-TCDD metabolism in isolated hepatocytes, demonstrating that 2,3,7,8-TCDD can induce its own rate of metabolism. Beatty et al (1978) also found a correlation between hepatic mixed-function oxidase (MFO) activity and the toxicity of 2,3,7,8-TCDD in rats. In both naturally occurring age and sex-related differences in MFO activity, and following administration of inducers and inhibitors of MFO enzyme systems, hepatic MFO activity was directly correlated with the 20-day LD_{50}.

Olson and Bittner (1983) reported that the rate of 2,3,7,8-TCDD metabolite formation in vitro was higher in hepatocytes from the hamster than in hepatocytes from the rat. Qualitative evaluation of in vivo and in vitro metabolites by HPLC also suggested significant interspecies variability. The authors suggested that such differences in metabolism may partially explain the differences in toxicity among species.

Excretion

The following discussion assumes that elimination is a first order process. With the exception of the guinea pig, which may follow zero order kinetics (Gasiewicz and Neal, 1979), elimination data yield a straight line on a semilogarithmic plot, indicating that elimination is a single, first order process. Hiles and Bruce (1976) have pointed out that the studies of Allen et al. (1975) and Piper et al. (1973) can be interpreted equally well by either zero or first order kinetics. The majority of the data, however, seem to support the assumption of a first order elimination process.

The excretion of 2,3,7,8-TCDD and its metabolites has been investigated in a number of species. Table III-3 summarizes results on the elimination of 2,3,7,8-TCDD-derived radioactivity, following a single exposure to ^{3}H- or [^{14}C]-2,3,7,8-TCDD. These studies show that 2,3,7,8-TCDD was slowly excreted from the bodies of all species tested, with a half-life in the body of 10-43 days. In the Syrian Golden hamster, the least sensitive mammalian species to the acute toxicity of 2,3,7,8-TCDD, excretion occurred readily through both the urine (35% of administered dose, 41% of total excreted radioactivity) and feces (50% of the administered dose, 59% of total excreted radioactivity) (Olson et al., 1980b; Gasiewicz et al., 1983a). The high levels found in the urine of infant monkeys were probably due to the incomplete separation of urine and feces (Van Miller et al., 1976). In all the other species tested so far, excretion occurred mainly through the feces (80-100% of total urinary and fecal radioactivity) with only minor amounts of 2,3,7,8-TCDD metabolites found in the urine (Piper et al., 1973; Allen et al., 1975; Rose et al., 1976; Gasiewicz and Neal, 1979). Only Piper et al. (1973) reported the excretion of metabolites in the expired air. During 21 days following administration of a single oral dose of [^{14}C]2,3,7,8-TCDD to rats, 3.2% of the administered radioactivity (4.6% of the excreted radioactivity) was recovered in the expired air.

TABLE III-3

Elimination of 2,3,7,8-TCDD

Species	Single Treatment µg/kg (route)	Half-Life for Elimination (days)	Relative % of TCDD-Derived Radioactivity		Reference
			Feces	Urine	
Guinea pig	2 (i.p.)	30.2 ± 5.8	94.0	6.0	Gasiewicz and Neal, 1979
Guinea pig	1.45 (oral)	22 - 43	NT	NT	Nolan et al., 1979
Rat	1.0 (oral)	31 ± 6	>99	<1	Rose et al., 1976
Rat	50 (oral)	17.4 ± 5.6	80.0	20.0	Piper et al., 1973
Rat	50 (oral)	21.3 ± 2.9	95.5	4.5	Allen et al., 1975
Rat	400 (i.p.)	NT	91.0	9.0	Van Miller et al., 1976
Monkey (adult)	400 (i.p.)	NT	78.0	22.0	Van Miller et al., 1976
Monkey (infant)	400 (i.p.)	NT	39.0	61.0	Van Miller et al., 1976
Mouse					
C57Bl/65	10 (i.p.)	11.0 ± 1.2	72.0	28.0	Gasiewicz et al., 1983a,b
DBA/2J	10 (i.p.)	24.4 ± 1.0	54.0	46.0	Gasiewicz et al., 1983a,b
B6D2F$_1$/J*	10 (i.p.)	12.6 ± 0.8	72.0	28.0	Gasiewicz et al., 1983a,b
Hamster	650 (i.p.)	10.8 ± 2.4	59.0	41.0	Olson et al., 1980a
Hamster	650 (oral)	15.0 ± 2.5	NT	NT	Olson et al., 1980a

*Offspring of C57Bl/6J and DBA/2J which are heterozygous at the Ah locus

NT = Not tested

Rose et al. (1976) investigated the elimination of [^{14}C]2,3,7,8-TCDD in rats given repeated oral doses of 0.01, 0.1 or 1.0 μg/kg/day Monday through Friday for 7 weeks, or a single dose of 1.0 μg/kg. In the single-dose study, no ^{14}C was excreted in the urine or expired air; in the repeated-dose study, however, 3-18% of the cumulative dose was excreted in the urine by 7 weeks. This study indicated that steady-state concentrations will be reached in the bodies of rats in ~13 weeks. The rate constant defining the approach to steady-state concentrations was independent of the dosage of 2,3,7,8-TCDD over the range studied. This is consistent with the observations of Fries and Marrow (1975) who found that the total retention in the bodies of rats was proportional to total intake. When rats were maintained on a diet containing either 7 or 20 ppb 2,3,7,8-TCDD, the amount of 2,3,7,8-TCDD retained in the body was 5.5 times the daily intake of 2,3,7,8-TCDD at 14 days, 7.5 times the daily intake at 28 days, and 10.0 times the daily intake at 42 days.

The data in Table III-3 suggest some interspecies differences in the half-life for elimination (t 1/2) of 2,3,7,8-TCDD. In the hamster, the least sensitive species to the acute toxicity of 2,3,7,8-TCDD, a mean t 1/2 of 10.8 days was observed (Olson et al., 1980a,b), and in the guinea pig, the most sensitive species to the acute toxicity of 2,3,7,8-TCDD, the mean t 1/2 was 30.2 days (Gasiewicz and Neal, 1979). The observed interspecies differences in the t 1/2 of 2,3,7,8-TCDD may in part be related to the relative sensitivity of a given species to the acute toxicity of 2,3,7,8-TCDD.

The intraspecies differences in the t 1/2 of 2,3,7,8-TCDD in three mouse strains may be due to the finding that the DBA/2J strain possesses ~2-fold greater adipose tissue stores than the C57Bl/6J and $B6D2F_1$/J strains (Gasiewicz et al., 1983b). The sequestering of the lipophilic toxin in adipose tissue stores of the DBA/2J mouse may contribute to the greater persistence of 2,3,7,8-TCDD in this strain.

In all of the rat studies shown in Table III-3, urinary and fecal elimination were monitored for a period of only 20-22 days, and from these data it was assumed that elimination followed a single component, first order kinetic model. Recently, Olson and Bittner (1983) examined the elimination of 2,3,7,8-TCDD-derived radioactivity in rats over a 35-day period following a single i.p. exposure at 1 μg ^{3}H-2,3,7,8-TCDD/kg. They observed first order kinetics for elimination, with a fast component having a t 1/2 of 7 days (representing 13% of total elimination) and a slow component having a t 1/2 of 75 days (87% of total). The second, slow component for elimination was evident only when urinary and fecal elimination were monitored for >30 days. This study suggests that 2,3,7,8-TCDD may be more persistent than earlier studies suggested. A preliminary study in the rhesus monkey indicates that 2,3,7,8-TCDD may be exceptionally persistent in adipose tissue. McNulty et al. (1982) estimated the apparent half-life of 2,3,7,8-TCDD in the fat of a monkey to be ~1 year.

Studies in the rat, guinea pig, hamster and mouse have found that all of the 2,3,7,8-TCDD derived radioactivity excreted in the urine and bile corresponds to metabolites of 2,3,7,8-TCDD (Olson et al., 1983). The apparent absence of 2,3,7,8-TCDD metabolites in liver and fat suggests that, once formed, the metabolites of 2,3,7,8-TCDD are readily excreted. Thus, urinary and biliary elimination of 2,3,7,8-TCDD is dependent upon metabolism of the toxin. Although urine and bile appear to be free of unmetabolized 2,3,7,8-TCDD, data from the hamster and rat indicate that a significant amount (10-40%) of unchanged 2,3,7,8-TCDD may be excreted into the feces (Olson et al., 1983). Unmetabolized 2,3,7,8-TCDD thus appears to enter the intestinal lumen by some route other than bile (direct intestinal elimination) for a number of days following treatment. Studies in lactating rats have also found that unchanged 2,3,7,8-TCDD may be excreted in the milk of lactating animals (Moore et al., 1976; Lucier et al., 1975). Lactation, direct intestinal elimination, and perhaps sebum may serve as routes for excretion of 2,3,7,8-TCDD, which are not dependent upon metabolism of the toxin. These data suggest that the _in vivo_ half-life for elimination of 2,3,7,8- TCDD may not directly reflect the rate of 2,3,7,8-TCDD metabolism in a given animal (Neal et al., 1982).

Summary

The toxicokinetics of 2,3,7,8-TCDD has been investigated in a number of laboratory animals; the reader is referred to recent reviews (Neal et al., 1982; Gasiewicz et al., 1983a; Olson et al., 1983) for indepth discussions of this subject.

Because 2,3,7,8-TCDD is a strongly lipophilic compound (Crummett and Stehl, 1973), gavage treatment with single or repeated doses of the compound in oil has resulted in absorption of ~50% of the dose administered to guinea pigs (Nolan et al., 1979), ~70-83% of the dose administered to rats (Rose et al., 1976; Piper et al., 1973) or to hamsters (Olson et al., 1980a).

Dietary administration of comparable dose-ranges to rats resulted in somewhat reduced GI absorption (~50-60% of administered dose was absorbed) (Fries and Marrow, 1975).

Using hepatic concentration of 2,3,7,8-TCDD as a endpoint, Poiger and Schlatter (1980) demonstrated a linear relationship in rats between the magnitude of an oral dose of 2,3,7,8-TCDD and absorption, up to a dose of ~1.0 μg. By treating rats with aqueous suspensions of soil treated with 2,3,7,8-TCDD these researchers (Poiger and Schlatter, 1980) were able to show a decrease in GI absorption of 2,3,7,8-TCDD directly proportional to the length of time the compound had been in contact with soil before the suspension was made and the rats were treated. Mixing 2,3,7,8-TCDD with a aqueous suspension of activated charcoal essentially eliminated absorption as measured by hepatic concentrations of 2,3,7,8-TCDD (Poiger and Schlatter, 1980). That adsorbant materials may reduce the GI absorption of 2,3,7,8-TCDD was observed by Van den Berg et al. (1983), who demonstrated that absorption of PCDDs was less from fly ash (naturally containing PCDDs) than from comparable doses of PCDDs from extracts of fly ash.

Percutaneous absorption of 2,3,7,8-TCDD has been estimated in rats to be ~40% of the absorption of an equivalent dose orally administered (Poiger and Schlatter, 1980). Dermal application of the compound in vaseline, polyethylene glycol or soil/water paste substantially reduced dermal absorption. Application of the compound in activated carbon/water paste virtually eliminated absorption.

Tissue distribution following oral or i.p. administration of 2,3,7,8-TCDD to rats appears to be preferentially to the liver and adipose tissue (Fries and Marrow, 1975; Rose et al., 1976; Van Miller et al., 1976; Kociba et al., 1978a). Other tissues showed substantially lower concentrations of 2,3,7,8-TCDD. Soon after treatment the liver may have concentrations ≈3 (Kociba et al., 1978a) to 5 (Rose et al., 1976) times that in adipose tissue. It was suggested that male rats accumulate 2,3,7,8-TCDD in the liver more efficiently than female rats (Fries and Marrow, 1975). Tissue distribution in mice (Manara et al., 1982) and hamsters (Olson et al., 1980a) seems to be similar to that in rats.

Monkeys, however, appear to accumulate 2,3,7,8-TCDD preferentially in adipose tissue > liver (Van Miller et al., 1976; McNulty et al., 1982). Two years after a single oral dose to a monkey, the fat contained 100 ppt and the liver 15 ppt 2,3,7,8-TCDD (McNulty et al., 1982). Prolonged tissue retention of the compound was thus demonstrated. Tissue distribution in guinea pigs appears similar to that in monkeys (Gasiewicz and Neal, 1979; Nolan et al., 1979) in that tissue levels in fat exceed those in the liver.

Evidence that 2,3,7,8-TCDD accumulates in the adipose tissue of exposed humans was presented by Young et al. (1983) who reported levels of 3-99 ppt in the adipose tissue of armed forces veterans claiming health problems related to Agent Orange.

Distribution of 2,3,7,8-TCDD to the fetus has been studied in rats (Moore et al., 1976) and mice (Nau and Bass, 1981; Nau et al., 1982). Levels of 2,3,7,8-TCDD were low in rat fetuses on gestation days 14 and 18 and appeared to be evenly distributed in all fetal tissues. At gestation day 21, the fetal liver showed a marked affinity for 2,3,7,8-TCDD (Moore et al., 1976). 2,3,7,8-TCDD was distributed to the fetuses of mice following oral, i.p. or s.c. administration (Nau et al., 1982). Maximum fetal concentrations occurred on gestation days 9 and 10; lower fetal concentrations were observed on gestation days 11-18, coincident with placentation. The fetal liver had less affinity for the compound that did the maternal liver.

In an early metabolism study, Vinopal and Casida (1973) reported that _in vivo_ or _in vitro_ studies with mice showed that polar metabolites of 2,3,7,8-TCDD were not produced by this species. In rats, however, hydroxylation and conjugation with glucuronide and sulfate have been demonstrated (Poiger and Schlatter, 1979; Olson et al., 1983; Poiger et al., 1982a. Glucuronide conjugates tended to predominate in the bile (Poiger and Schlatter, 1979) and sulfate conjugates were located in the urine (Olson et al., 1983).

Poiger and Schlatter (1979) stated that liver metabolism of 2,3,7,8-TCDD proceeds slowly in the liver. Neal et al. (1982) demonstrated that the rate of hepatic metabolism was enhanced by activated cytochrome P-450 monooxygenase. It was suggested that metabolism of 2,3,7,8-TCDD proceeds by the formation of reactive epoxide intermediates (Poland and Glover, 1979). That dechlorination also occurs was demonstrated by Olson et al. (1983) and Sawahata et al. (1982) who identified tri- and dichlorodibenzo-_p_-dioxins as metabolites in _in vitro_ rat hepatocyte systems. From the bile of dogs, six major metabolites have been identified (Poiger et al., 1982a); hydroxylated conjugates of tetra-, tri- and dichlorodibenzo-_p_-dioxin predominated.

When the excretion data are subjected to a semi-logarithmic plot, a straight line results, suggesting that elimination of 2,3,7,8-TCDD is a first-order phenomenon, particularly for rats. Excretion in the guinea pig may be a zero-order process (Gasiewicz and Neal, 1979). The half-life for body elimination varied considerably with ranges of ~10-15 days in the hamster (Olson et al., 1980a), the species least sensitive to the toxic effects of 2,3,7,8-TCDD, to estimates of ~11-24 days in the mouse (Gasiewicz et al., 1983a,b), ~17-31 days in the rat (Rose et al., 1976; Piper et al., 1973; Allen et al., 1975) and ~22-30 days in the guinea pig (Gasiewicz and Neal, 1979; Nolan et al., 1979). One strain of mice, DBA/2J, had a half-life for elimination (~24 days) about twice as high as other strains tested by Gasiewicz et al. (1983a,b). These authors also noted that this strain of mice accumulated 2,3,7,8-TCDD in adipose tissue more strongly than other strains and that this phenomenon probably resulted in slowed body elimination. Half-lives for body elimination of 2,3,7,8-TCDD have not been calculated for the monkey, but it was suggested that the tendency of this species to accumulate 2,3,7,8-TCDD in body fat may well result in slowed body elimination (Van Miller et al., 1976).

Recently, Olson and Bittner (1983) examined the elimination of 2,3,7,8-TCDD in rats over a longer period than the studies previously summarized and determined that a biphasic elimination occurred. They suggested a half-life of ~7 days for the initial rapid phase and a half-life of ~75 days for the slower phase, probably related to release from stores of body fat. McNulty et al. (1982) estimated the half-life for elimination from the fat of monkeys to be ~1 year.

The fecal route seems to be the major pathway for the elimination of 2,3,7,8-TCDD-derived radioactivity from rats (Rose et al., 1976; Piper et al., 1973; Allen et al., 1975; Van Miller et al., 1976), guinea pigs (Gasiewicz and Neal, 1979) and mice (Gasiewicz et al., 1983a,b). Urinary excretion played less of a role in these species, accounting for <1-28% of total excreted radioactivity while fecal excretion accounted for 72->99% of the eliminated radioactivity. Urinary excretion accounted for a more substantial proportion of body elimination in hamsters (41% compared to 59% by feces) (Olson et al., 1980a) and that strain of mice (DBA/2J) which preferentially accumulated 2,3,7,8-TCDD in body fat (Gasiewicz et al., 1983a,b).

The failure to detect metabolites of 2,3,7,8-TCDD in liver and fat (Olson et al., 1983) indicates that elimination of the metabolites occurs rapidly and that the rate of elimination is governed primarily by the rate of hepatic metabolism.

IV. HUMAN EXPOSURE

Humans may be exposed to chemicals such as dioxin from a variety of sources, including drinking water, food and ambient air, occupational settings and consumer products. This analysis of human exposure to dioxin is limited to drinking water, food and ambient air because those media are considered to be sources common to all individuals. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and on several factors over which there is little control. Where one lives, works and travels, what one eats, and physiologic characteristics related to age, sex and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

In the Exposure Estimation Section of this chapter, available information is presented on the range of levels of human exposure and intake for dioxin from drinking water, food and ambient air for the 70 kg adult male. It is not possible to provide an estimate of the number of individuals experiencing specific combinations of intake from those three sources. However, the Summary Section of this chapter provides some insight into the relative contributions of the three sources, especially drinking water, for the range of intake values suggested by the available data.

Exposure Estimation

Drinking Water. No information was found in either the Federal surveys or state data on measured levels of dioxins in drinking water from community water supplies.

Diet. Limited information was obtained on the occurrence of dioxins in food. Dioxins are not analyzed for in the Food and Drug Administration's Market Basket Studies. However, a few studies address the occurrence of dioxin [in particular, the occurrence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD)] in specific foods.

The Ambient Water Quality Criteria Document for 2,3,7,8-tetrachlorodibenzo-p-dioxin (U.S. EPA, 1984) reported studies conducted by others for dioxin (2,3,7,8-TCDD) determinations in food. In a study by Jensen et al. (1983), rice grain from fields in Arkansas, Louisiana and Texas were analyzed after the application of 2,4,5-T (containing 0.4 mg/kg 2,3,7,8-TCDD). None of the grain analyzed contained 2,3,7,8-TCDD (detection limit = 2-10 ng/kg), nor was any found in 30 samples of rice bought in retail stores throughout the United States. Other studies on the contamination of fruits, vegetables or grains in the U.S. with 2,3,7,8-TCDD have apparently not been conducted (U.S. EPA, 1984).

Levels of 2,3,7,8-TCDD in the fat of cattle that grazed on pasture treated with 2,4,5-T ranged from 4-70 ng/kg in one study (U.S. EPA, 1980). In another investigation by Kocher et al. (1978), levels were reported as undetected (detection limit = 1 ng/kg). The number of samples were not reported for either study.

Bumb et al. (1980) analyzed charcoal-broiled steak to determine if 2,3,7,8-TCDD was formed due to the broiling process. TCDD was not detected (detection limit = 1-10 ng/kg).

2,3,7,8-TCDD has been detected in many species of commercial and noncommercial fish in several rivers and lakes in the United States (U.S. EPA, 1984). Levels ranged from 1-695 ng/kg (including all sites and types of fish studied). The maximum daily intake of 2,3,7,8-TCDD was estimated by Cordle (1983) for residents of the Great Lakes region who regularly consume fish from the Great Lakes. The intake ranged from 0.39-8.4 ng/day.

Bovine milk surveillance samples have been analyzed for 2,3,7,8-TCDD (detection limit = 1 ng/kg) by several investigators after normal application of 2,4,5-T on pasture in Oklahoma, Arkansas and Missouri, as well as from quarantined milk samples in Michigan (U.S. EPA, 1984). 2,3,7,8-TCDD was undetected in all samples.

A study of 103 samples of breast milk from mothers living in sprayed areas of the United States (U.S. EPA, 1980) reported no detection of 2,3,7,8-TCDD (detection limit = 1-4 ng/kg). About six of nine human milk samples analyzed by Langhorst and Shadoff (1980) may have contained 2,3,7,8-TCDD at levels slightly higher than the detection limits (0.2-0.7 ng/kg). However, due to lack of information on the accuracy of the data, it was concluded by those authors that 2,3,7,8-TCDD was not present.

The available studies on the occurrence of dioxins in food do not represent the food groups that comprise an average diet. Since no information was obtained on most food groups, it was impossible to calculate dietary intake. Consequently, the contribution of dietary intake to total exposure of dioxins could not be assessed.

Air. Information on inhalation exposure to dioxins is limited. Dioxins usually exhibit very low vapor pressures and, therefore, tend not to be found in ambient air (Cleverly, 1984). No data on inhalation exposure of dioxins is currently available, however, some other studies as reported in U.S. EPA (1984) are cited below.

Nash and Beall (1980) reported very low air levels of 2,3,7,8-TCDD following the application of emulsified and granular Silvex. Levels were reported to range between 0.09×10^{-6} ng/m^3 and 7.98×10^{-2} ng/m^3.

Air filter samples collected after an industrial fire were analyzed for 2,3,7,8-TCDD by Harvan et al. (1981). Of nine samples analyzed, one contained 0.02 ng/m^3 of 2,3,7,8-TCDD, four contained <0.009 ng/m^3 of 2,3,7,8-TCDD and four (as reported in U.S. EPA, 1984) "probably" contained 0.005-0.012 ng/m^3 of unspecified TCDD isomer (not the 2,3,7,8-TCDD isomer).

Air levels near two hazardous waste sites have been monitored. 2,3,7,8-TCDD was not detected near Love Canal at a detection limit of 1-20 ng/kg (U.S. EPA, 1982). However, at a disposal site near Jacksonville, Arkansas, Thibodeaux (1983) reported an average concentration of 1100 ng/kg of 2,3,7,8-TCDD in two air particulate samples.

Atmospheric emissions from municipal incinerators and from certain fires are other sources of polychlorinated dioxins. Quantities emitted may vary due to differing incinerator efficiencies, and the quantity measured depends on the capability to reliably characterize such emissions. Bumb et al.

(1980) and Buser and Rappe (1980) detected 0.4 ng/g of 2,3,7,8-TCDD in fly ash from a United States municipal incinerator (location not given in U.S. EPA, 1984).

At an industrial boiler in the U.S. where pentachlorophenol was known to have been burned (location not given), Rappe et al. (1983) reported ~5 mg/kg of polychlorinated dibenzo-p-dioxins (PCDDs) in the baghouse and bottom ash. Among the many isomers found, only a small amout of 2,3,7,8-TCDD could be quantified (quantification not reported in U.S. EPA, 1984).

Soot samples from a transformer fire in Binghamton, NY were analyzed and revealed that 2,3,7,8-TCDD and 1,2,3,7,8-pentachlorodibenzodioxin (1,2,3,7,8-penta-CDD) were the predominate isomers of the PCDDs formed (Buser and Rappe, 1983; Rappe et al., 1983). 2,3,7,8-TCDD was quantified at 0.6 mg/kg, 1,2,3,7,8-penta-CDD was not quantified. Analyses of wipe tests from a garage adjacent to this accident site showed the presence of PCDDs prior to the cleaning of the garage (Tiernan et al., 1982; Tiernan, 1983).

Summary

Sufficient information was not available to assess the extent of human exposure to dioxins or to determine the relative source contribution to total exposure by the three common media (drinking water, food and ambient air).

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V. HEALTH EFFECTS IN ANIMALS

Experimental Animals

Acute Toxicity.

Lethal Effects -- There have been studies in a variety of species defining the doses necessary to cause death after acute exposure to 2,3,7,8-TCDD. A summary of the single dose LD_{50} data for 2,3,7,8-TCDD is presented in Table V-1. The dose that results in death varies extensively with species, with the male guinea pig the most sensitive species tested (LD_{50} of 0.6 μg/kg) (Schwetz et al., 1973), and the male hamster the least sensitive species tested (LD_{50} of 5051 μg/kg) (Henck et al., 1981). The rat and monkey appear to be the second most sensitive species, with LD_{50}s between 22 and 70 μg/kg (Schwetz et al., 1973; McConnell et al., 1978a), while other species tested (rabbit and mouse) had LD_{50}s between 114 and 283 μg/kg (Schwetz et al., 1973; McConnell et al., 1978b; Vos et al., 1974). Schwetz et al. (1973) found male rats more sensitive to 2,3,7,8-TCDD, while Beatty et al. (1978) found adult female and weanling male rats more sensitive than adult male rats (see Table V-1). In C57Bl/10 mice, Smith et al. (1981) reported adult males to be far more sensitive to the acute toxicity of 2,3,7,8-TCDD than adult females. Thus, data on sex differences in sensitivity to the acute toxicity of 2,3,7,8-TCDD are conflicting and may depend on the species examined.

Harris et al. (1973) studied the toxic effects of 2,3,7,8-TCDD in rats, mice and guinea pigs with regard to single or multiple exposures. Similar effects were observed after a single exposure to 2,3,7,8-TCDD as were observed when multiple exposures totaled the same dose as received in the single exposure. As illustrated most clearly in rats, a single dose of

TABLE V-1

Lethal Doses of 2,3,7,8-TCDD Following Acute Exposure

Species/Strain	Sex/No./Group	Route/ Vehicle	Dose Tested (µg/kg)	Duration of Observation	LD_{50} (µg/kg)	Comments	Reference
Guinea pigs/ Hartley	M/NR	gavage/corn oil-acetone (9:1)	NR	2-8 weeks	0.6 (0.4-0.9)*	Time to death was 5-34 days, the 2,3,7,8-TCDD was 91% pure	Schwetz et al., 1973
Guinea pigs/ Hartley	M/NR	gavage/corn oil-acetone (9:1)	NR	2-8 weeks	2.1 (1.5-3)*	Time to death was 9-42 days, the 2,3,7,8-TCDD was 99% pure	Schwetz et al., 1973
Guinea pigs/ Hartley	M/9	gavage/ corn oil	NR	30 days	2	Median time to death was 17-20 days, marked weight loss, thymus atrophy, intestinal hemorrhage, no porphyria and only mild liver injury	McConnell et al., 1978b
Guinea pigs/ Hartley	F/6	gavage/ corn oil	0.1 0.5 2.5 12.5 20.0	42 days	2.5 (1.2-5.4, 95% confidence)	Time to first death was 32 days in the 2.5 µg/kg group, with 50% mortality by day 42	Silkworth et al., 1982
Guinea pigs/ Hartley	F/6	gavage/ methyl cellulose	0.1 0.5 2.5 12.5 20.0	12 days	19 (15-23, 95% confidence)	Time to first death was 12 days in the 20.0 µg/kg group, with 67% mortality by day 42	Silkworth et al., 1982
Rats/ Sherman	M/5-10	gavage/corn oil-acetone (9:1)	8 16 32 63	2-8 weeks	22	Time to death was 9-27 days, the 2,3,7,8-TCDD was 91% pure	Schwetz et al., 1973
Rats/ Sherman	F/NR	gavage/corn oil-acetone (9:1)	NR	2-8 weeks	45 (30-66)*	Time to death was 13-43 days, the 2,3,7,8-TCDD was 91% pure	Schwetz et al., 1973
Rats/Sprague-Dawley	M/6	i.p./olive oil	NR	20 days	60	LD_{50} (µg/kg, mean ± SE) adult male, 60.2 ± 7.8; weanling male, 25.2 ± 1.4	Beatty et al., 1978
Rats/Sprague-Dawley	F/6	i.p./olive oil	NR	20 days	25	Adult female had a mean ± SE of 24.6 ± 2.0 µg/kg	Beatty et al., 1978

TABLE V-1 (cont.)

Species/Strain	Sex/No./Group	Route/ Vehicle	Dose Tested (µg/kg)	Duration of Observation	LD_{50} (µg/kg)	Comments	Reference
Monkey/rhesus	F/3	gavage/ corn oil	0 70 350	>35 days	<70	Weight loss, edema, severe thymus atrophy, loss of hair, mild liver damage	McConnell et al., 1978a
Mice/C57Bl	M/14	gavage/corn oil-acetone (9:1)	0 100 150 200	60 days	114	Time to death in the high dose group was 15-20 days, bw loss, edema in 25% of treated animals, severe thymic and spleen atrophy, hemorrhage in the region of the eye and small intestine, liver necrosis in the centrilobular region	Vos et al., 1974
Mice/C57Bl	M/9	gavage/ corn oil	NR	30 days	283.7	Median time to death was 22-25 days, dose-related bw loss, thymic atrophy, increased liver weight and porphyria, gross and histeric liver alterations, subcutaneous edema, intestinal hemorrhage	McConnell et al., 1978b
Mice/C57Bl/10	M/5	gauge/ arachis oil	85 107 135 170 213	45 days	146	95% confidence limits of 111-211 µg/kg. Most deaths occurred from 22-26 days after dosing. Signs of porphyria, edema, hemorrhage.	Smith et al., 1981
Mice/C57Bl/10	F/5	gauge/ arachis oil	85 107 135 170 213 269 338 426 536	45 days	>450	1 of 4 animals died at dose of 426 µg/kg	Smith et al., 1981
Mice/C57Bl/6J	M/NR	i.p./olive oil	NR	30 days	132	BGD2F_1/J mice are the offspring of C57Bl/6J and DBA/2J.	Gasiewicz et al., 1983a,b
Mice/DBA/2J	M/NR	i.p./olive oil	NR	30 days	620	The BGD2_1/J mice are heterozygous at the Ah locus.	Gasiewicz et al., 1983a,b
Mice/BGD2F_1/J	M/NR	i.p./olive oil	NR	30 days	300	No comment	Gasiewicz et al., 1983a,b

TABLE V-1 (cont.)

Species/Strain	Sex/No./Group	Route/ Vehicle	Dose Tested (µg/kg)	Duration of Observation	LD_{50} (µg/kg)	Comments	Reference
Rabbits/ New Zealand	M&F/NR	gavage/corn oil-acetone (9:1)	NR	2-8 weeks	115 (38-345)*	Time to death was 6-39 days, the 2,3,7,8-TCDD was 91% pure	Schwetz et al., 1973
Rabbits/ New Zealand	M&F/5	i.p./ corn oil	32 63 126 252 500	4 weeks	NR	Time to death was 6-23 days, 2-3 animals/group died in all but the low exposure group	Schwetz et al., 1973
Rabbits/ New Zealand	M&F/NR	dermal/ acetone	31.6 63 126 252 500	3 weeks	275 (142-531)*	Time to death was 12-22 days	Schwetz et al., 1973
Hamster/ golden Syrian	M/6	gavage/corn oil-acetone (9:1)	0 300 600 1000 3000 6000	55 days	5051 (3876-10,487, 95% confidence)	Time to death was 26-43 days, the liver and thymus appeared to be the primary target organs, only 1 death occurred in the 300 and 3000 µg/kg group	Henck et al., 1981
Hamster/ golden Syrian	M&F/5-6	i.p./ olive oil	0 500 1000 2000 3000	50 days	>3000	Significant, dose-related decrease in thymus weight starting at 500 µg/kg, only 2 deaths occurred out of 11 hamsters in the 3000 µg/kg group.	Olson et al., 1980b
Hamster/ golden Syrian	M/5	gavage/ olive oil	500 1000 2000 3000	50 days	1157	Death generally occurred between 24 and 45 days, decrease in bw above 2000 µg/kg, proliferative ileitis with mild to severe inflammation	Olson et al., 1980b
Dogs/beagle	M/2	gavage/corn oil-acetone (9:1)	3000	2-8 weeks	NA	All animals died	Schwetz et al., 1973
Dogs/beagle	F/2	gavage/corn oil-acetone (9:1)	30 100	2-8 weeks	NA	All animals survived	Schwetz et al., 1973

*The number in parentheses appears to indicate the range of lethal doses; however, the article did not specify what these numbers represented.

i.p. = intraperitoneal; NR = Not reported; NA = Not applicable

25 µg/kg, 6 weekly doses of 5 µg/kg, or 30 daily doses of 1 µg/kg were all the threshold dose for observing a decrease in body weight. Other endpoints, including lethality, decrease in thymus weight, and a no effect level for body weight change in rats, mice and guinea pigs in general appeared likewise to require a specific threshold level regardless of whether this level was achieved through a single exposure or a small number of multiple exposures.

Although 2,3,7,8-TCDD has over a 10^3-fold difference in toxicity depending upon the species tested, some of the signs of lethal toxicity were the same regardless of species. One of the most characteristic observations after acute lethal exposure to 2,3,7,8-TCDD was the protracted time between exposure and death (see Table V-1). In determining the LD_{50} in the least sensitive animal, the hamster, the test animals died between 24 and 45 days after a single acute exposure (Olson et al., 1980b), and similar observations were made in all other species tested including the most sensitive species, the guinea pig, in which animals died up to 42 days after treatment (Schwetz et al., 1973).

During this extended period between treatment and death the animals had poor weight gain or loss of weight and appeared to be "wasting away." In female Wistar rats intubated with 2,3,7,8-TCDD at a dose of 100 µg/kg, the weight loss was biphasic (Courtney et al., 1978). The initial weight loss occurred rapidly during the first 7-10 days after treatment and was associated with decreased food and water consumption. This initial phase of weight loss was reversed with the resumption of normal food intake for 4 or 5 days, only to be followed by a second, more gradual, decline in food and water intake and weight until death. Providing animals with an adequately nutritious liquid diet by intubation did not appreciably alter the pattern of weight loss nor affect survival. In contrast, Gasiewicz et al. (1980) observed that providing rats with total parenteral nutrition would prevent some of the weight loss induced by 2,3,7,8-TCDD; however, there was no protection from the lethal effects of 2,3,7,8-TCDD. In yet another study, Seefeld and Peterson (1983) suggest that a reduction in food intake caused by 2,3,7,8-TCDD is primarily responsible for the loss of body weight or depressed growth rate of rats. Pair-fed control rats lost weight at the same rate and to the same extent as their weight-matched 2,3,7,8-TCDD-treated partners (25 or 50 μg/kg) until day 10 after treatment. At 20-35 days after treatment, the body weight of the two groups began to diverge, with the pair-fed control group having body weights that were 20-30 g higher than the corresponding 2,3,7,8-TCDD groups. The mortality in the 25 and 50 μg/kg groups was 33 and 75%, respectively, while in the corresponding pair-fed groups the mortality was 0 and 15%. The authors proposed a hypothesis that 2,3,7,8-TCDD lowers a regulated level or "set-point" for body weight control in the rat. The ensuing change in food intake was thought to occur secondarily to the change in set-point.

Also, severe thymic atrophy is universally observed in all species given lethal doses of 2,3,7,8-TCDD, and since weight loss and thymic atrophy are both associated with malnutrition, van Logten et al. (1981) investigated the effects of dietary protein on the toxicity of 2,3,7,8-TCDD. Groups of female Fischer 344 rats administered 2,3,7,8-TCDD (20 μg/kg) and maintained on low (3.5%), normal (26%) or high (55%) protein diets maintained approximately the same amount of weight (-0.2±3, 7±6 and 7±3 g for each dietary group, respectively) during the subsequent 10-day period. The weight gain in treated animals was 10-18 g less than that in the respective control rats. Dietary protein also had no effect on preventing or enhancing the 2,3,7,8-TCDD induced thymic atrophy. Although weight loss and thymic atrophy were present in most species tested, there were other symptoms which were characteristic of toxicity in only some species.

In the guinea pig, besides thymic atrophy, no gross changes were observed in internal organs after a lethal oral or i.p. dose of 2,3,7,8-TCDD (Greig et al., 1973, Gupta et al., 1973). Hemorrhages were observed in a number of organs including the adrenal gland, urinary bladder, GI tract and mesenteric lymph nodes; however, these were considered unremarkable changes by Gupta et al. (1973). Histologic examination confirmed the gross observations with atrophy and lymphoid cell depletion in the thymus, spleen and lymph nodes, and hemorrhages observed in many organs. In addition, marked hyperplasia of the urinary bladder was observed. Of particular interest was the absence of severe toxic effects on the liver. Gross observation under UV light indicated no excess of porphyrin, while histologic examinations revealed diffuse single cell necrosis. Identical observations were made by McConnell et al. (1978b) in guinea pigs administered lethal doses of 2,3,7,8-TCDD, with the additional observation that the sternal bone marrow was hypocellular in all types of blood-forming cells.

Turner and Collins (1983) described some histologic changes in the liver of guinea pigs treated with 2,3,7,8-TCDD. Groups consisting of 4-6 female Hartley guinea pigs were treated with 2,3,7,8-TCDD at doses of 0.0, 0.1, 0.5, 2.5, 12.5 or 20 μg/kg, and 1 male guinea pig each was treated with a dose of 0.1 or 0.5 μg/kg. The 2,3,7,8-TCDD was administered by gavage as an aqueous suspension in 0.75% methyl cellulose and surviving animals were killed 42 days after treatment. A second group of guinea pigs (6 males and 6 females/dose) were administered soot generated from a fire in a transformer cooled by polychlorinated biphenyls and chlorinated benzenes (1, 10, 100 and 500 mg/kg). The histologic observations as described were applied in general to both treatment groups and there was no apparent relationship between dose and response. The authors reported that, compared to controls, all experimental groups showed liver alterations but that qualitative differences among the dosage groups were not detectable by light microscopy. At the light microscope level, hepatocellular hypertrophy, steatosis, focal necrosis, cytoplasmic degeneration and acidophilic hyalin-like cytoplasmic inclusion bodies were observed. Even though there was no dose-response relationship for these liver lesions, the doses spanned a range that resulted in the lowest dose being nonlethal (none of the 4 female guinea pigs died during the study), while in the high dose group 4 of 6 animals died before 42 days post-treatment. The LD_{50} for female guinea pigs was determined in this study, and reported by Silkworth et al. (1982), to be 2.5 or 19 μg/kg bw depending on whether the compound was administered by gavage in corn oil or in aqueous methyl cellulose.

The greatest difference at necropsy in the gross and histologic effects in rats and mice of exposure to lethal doses of 2,3,7,8-TCDD was pathologic alterations in the liver, as compared with guinea pigs. An early report by Buu-Hoi et al. (1972) described alterations in the architecture of the liver of rats within 5 days of receiving a low dose of 2,3,7,8-TCDD (10 μg/kg by i.p. injection). At higher oral doses of 100 or 50 μg/kg, which killed 43 and 7% of the animals, respectively, Gupta et al. (1973) also observed marked distortion of liver architecture in rats; however, only mild regenerative changes of the liver were observed at the sublethal dose of 5 µg/kg administered weekly for 6 weeks. Liver toxicity appeared to develop slowly in the rat with no change in liver function, as indicated by plasma protein and bilirubin levels, or alkaline phosphatase, glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activity being detected 3 days after intubation with 2,3,7,8-TCDD at a dose of 200 µg/kg (Greig et al., 1973). Bilirubin levels were, however, markedly elevated from 0.33 µg/100 mℓ in control animals to 10.97 µg/100 mℓ in treated animals 21 days after exposure (the other parameters were not measured at this time, although plasma protein was slightly but significantly decreased when determined 9 days post-treatment). As in rats, the livers of mice exposed to lethal levels of 2,3,7,8-TCDD had signs of necrotic changes (Vos et al., 1974); however, Jones and Grieg (1975) reported that the centrilobular necrosis, bile duct proliferation and lipid accumulation were more extreme in mice than in rats. Examination of mouse livers using long wave UV light showed fluorescence suggestive of excess porphyrin accumulation (McConnell et al., 1978b). Although excess porphyrins may be present in the livers from 2,3,7,8-TCDD-exposed rats, fluorescence is not usually observed.

Besides effects on the liver, 2,3,7,8-TCDD exposure produced other toxic effects in rats and mice that were not observed or were observed to a lesser extent in guinea pigs. In rats that died from 2,3,7,8-TCDD exposure, there were extensive hemorrhages of the heart, liver, brain, adrenal gland and GI tract along with ulcers and necrosis of the glandular stomach, and in females, atrophy of the uterus (Gupta et al., 1973). In mice, facial edema was severe and the testicles of males appeared degenerated with necrotic spermatocytes and spermatozoa present (McConnell et al., 1978b; Vos et al., 1974). Death in mice was frequently attributed to terminal hermorrhages (Vos et al., 1974).

In monkeys exposed to lethal levals of 2,3,7,8-TCDD, McConnell et al. (1978a) reported clinical and histologic signs of toxicity, some of which were similar to those already described for other species. Severe thymic atrophy and edema occurred in treated animals, as well as extensive weight loss that could account for up to 38% of the body mass. As in guinea pigs, liver injury appeared to be mild; however, increased serum GOT and aldolase activity and decreased albumin levels indicative of liver pathology occurred near the time of death. As observed in mice, the bone marrow of monkeys was hypocellular. In addition to the above signs of toxicity, which were observed in other species as well, monkeys had progressive loss of hair, toenails and fingernails, with associated dermatitis consisting of the development of a crusty texture to the skin, squamous metaplasia of sebaceous glands and gastric mucosal dysplasia. As with most other species, a specific cause of death could not be determined for monkeys. Poland and Knutson (1982) have summarized the toxic response of various species to 2,3,7,8-TCDD (Table V-2).

There was very little information on the lethal effects of PCDD congeners other than 2,3,7,8-TCDD. McConnell et al. (1978b) determined the LD_{50} for nine congeners of PCDD following a single treatment by gavage in mice and guinea pigs. A comparison of the LD_{50} expressed as μmol/kg body weight is presented in Table V-3. The limited data suggest that congeners containing chlorine in the 2,3,7,8 positions were more biologically

TABLE V-2

Toxic Responses Following Exposure to 2,3,7,8-TCDD: Species Differences[a]

	Monkey	Guinea Pig	Cow[b]	Rat	Mouse	Rabbit[b]	Chicken[b]	Hamster
Hyperplasia and/or metaplasia								
Gastric mucus	++[c]	0	+	0	0			0
Intestinal mucosa	+							++
Urinary tract	++	++	++	0	0			
Bile duct and/or gall bladder	++	0	+		++			0
Lung: focal alveolar				++				
Skin	++	0	+[d]	0	0	++		0
Hypoplasia, Atrophy or Necrosis								
Thymus	+	+	+	+	+		+	+
Bone marrow	+	+			±		+	
Testicle	+	+		+	+		+	
Other								
Liver lesions	+	±		++	+	++	+	±
Porphyria	0	0		+	++		+	0
Edema	+	0		0	+		++	+

[a] References: Monkey (McConnell et al., 1978b; Norback and Allen, 1973; Allen et al., 1977); guinea pig (McConnell et al., 1978b; McConnell, 1980; Moore et al., 1979; Turner and Collins, 1983); cow (McConnell, 1980); rat (McConnell, 1980; Kociba et al., 1978a,b, 1979); mouse (Schwetz et al., 1973; McConnell et al., 1978b; Vos et al., 1973); rabbit (Kimmig and Schultz, 1957; Schwetz et al., 1973; Vos and Beems, 1971); chicken (Schwetz et al., 1973; Norback and Allen, 1973; Allen and Lalich, 1962; Vos and Koeman, 1970); hamster (Olson et al., 1980b; Henck et al., 1981).

[b] Responses followed exposure to 2,3,7,8-TCDD or structurally related chlorinated aromatic hydrocarbons.

[c] Symbols: 0, lesion not observed; +, lesion observed (number of "+" denote severity); ±, lesion observed to a very limited extent; blank, no evidence reported in literature.

[d] Skin lesions in cattle are observed, but they differ from the skin lesions observed in other species.

Adapted from Poland and Knutson, 1982

TABLE V-3

Estimated Single Oral LD_{50-30} Values for PCDDs[a]

Chlorination of PCDDs	Guinea Pigs (μmol/kg)[b]	Mice (μmol/kg)[b]
2,8	>1180	NR
2,3,7	120.41	>10
2,3,7,8	0.006	0.88
1,2,3,7,8	0.009	0.94
1,2,4,7,8	3.15	>14
1,2,3,4,7,8	0.185	2.11
1,2,3,6,7,8	0.178-0.255[c]	3.19
1,2,3,7,8,9	0.153-0.255[c]	>3.67
1,2,3,4,6,7,8	>1.400	NR

[a] Source: Adapted from McConnell et al., 1978b

[b] Spearman-Karber method

[c] Estimated range due to variability in replicates

NR = Not reported

active than congeners deficient in a chlorine from any one of these positions. It also appears that addition of one or more chlorines to 2,3,7,8-TCDD results in a decrease in lethality. Although the congeners vary in effective dose between mice and guinea pigs, the relative order of toxicity of these congeners did not change. Also, similar effects of toxicity were observed for all congeners as described above for 2,3,7,8-TCDD when the comparison was made within a single species.

Effects on the Liver -- The histological and ultrastructural changes in the liver induced by oral exposure to 2,3,7,8-TCDD have been reported by Fowler et al. (1973), Jones and Butler (1974) and Jones (1975). Fowler et al. (1973) treated groups of 30 male rats with a single dose of 2,3,7,8-TCDD at 0.0, 5 and 25 μg/kg by gavage. The animals were killed in groups of 5 on days 1, 3, 6, 9, 16 and 28 after treatment and the livers were prepared for histologic examination. The major ultrastructural change observed was a dose-related increase in the smooth and rough endoplasmic reticulum (ER) in cells near the bile canaliculi. The initial increases appeared at day 3, with the maximal response occurring on days 6 and 9. By day 16 the smooth ER was nearly absent from the parenchymal cells, although large amounts of rough ER were still present. By day 28 the cells had returned to normal appearance. These changes in liver cells following 2,3,7,8-TCDD treatment would be consistent with the induction of protein and RNA synthesis.

Transmission electron microscopic observations revealed that single i.p. administration of 20 μg/kg of 2,3,7,8-TCDD in Sprague-Dawley male rats produces necrotizing hepatic lesions which become progressively worse up to the 16th week postexposure followed by gradual improvement of the condition and disappearance of the lesions (Weber et al., 1983).

At higher doses of 200 μg/kg, Jones and Butler (1974) observed necrosis and proliferative changes in the liver of rats to be the predominant lesions. After treatment by gavage, groups of 4 male and 4 female rats were killed and examined on a weekly basis for 10 weeks. By the first week, degenerating cells were observed near the central vein and these lesions progressed to areas of focal necrosis by the sixth week. Superimposed on the necrotic changes were hyperplasia of the viable cells with multinucleated cells common by the ninth week. At week 10 central vein fibrosis and scattered necrosis remained. Fine structure observed after this large dose of 2,3,7,8-TCDD also revealed increases in smooth ER; however, the most striking effect was degeneration of the plasma membrane with the resulting fusion of parenchymal cells. In a study of similar design, Jones (1975) followed the distribution with time after treatment of membrane associated ATPase activity by histochemical techniques. At 3 days after treatment, the first changes in ATPase patterns were observed, with loss of activity along the canalicular borders and some increased activity in the sinusoids. The midzonal and periportal zones had normal activity at this time. The loss of ATPase activity persisted for 34-42 days, and paralleled the histologic lesions described previously (Jones and Butler, 1974). In rats that survived treatment, the ATPase activity was back to normal by 9 months.

Peterson et al. (1979a) further studied the effect of 2,3,7,8-TCDD at lower doses on hepatocyte plasma membrane ATPase activity. Liver surface membranes (LSM) isolated from male Holtzman rats 2, 10, 20 or 40 days after intubation with 2,3,7,8-TCDD at 0.0, 10 or 25 μg/kg were used for determination of Na^+, K^+-ATPase and Mg^{++}-ATPase activity. The activity of Na^+, and K^+-ATPase was depressed to the same extent for both doses of

2,3,7,8-TCDD from day 2-40 after treatment, while a similar depression of the Mg^{++}-ATPase activity was observed only in the high dose group. In the low-dose group, there was a decrease in Mg^{++}-ATPase at 20 days, but recovery to normal levels occurred by 40 days post-treatment. It was demonstrated that the effect of 2,3,7,8-TCDD on ATPase activity was not the result of 2,3,7,8-TCDD induced food deprivation and _in vitro_ studies indicated that the loss of activity was not due to the direct interference of 2,3,7,8-TCDD with the enzyme. Quantitative changes (both increases and decreases) have been reported for the protein composition of plasma membranes isolated and analyzed by electrophoresis from Sprague-Dawley rats 10 days after an i.p. injection of 2,3,7,8-TCDD, indicating that exposure was actually affecting membrane components (Brewster et al., 1982).

Peterson et al. (1979a) did observe a positive correlation between the levels of LSM ATPase activity and both _in vivo_ cumulative biliary excretion of ouabain and bile flow (μℓ/min/g liver). Using perfused liver, however, Peterson et al. (1979b) reported a segregation between LSM ATPase activity and biliary excretion of ouabain when 2,3,7,8-TCDD rats were exposed to the protective agents pregnenolone-16α-carbonitrile or spironolactone. It was concluded that LSM ATPase did not directly participate in ouabain transport.

Additional studies have described the effect of 2,3,7,8-TCDD on the biliary excretion of a variety of xenobiotics. Early studies by Hwang (1973) investigated 2,3,7,8-TCDD inhibition of biliary excretion in male CD rats given a single dose of 2,3,7,8-TCDD at 25 or 5 μg/kg by gavage. Animals were examined for indocyanine green (ICG) excretion 1, 7 and 16 days after treatment. Unlike Peterson et al. (1979a), Hwang (1973) observed an inverse relationship between 2,3,7,8-TCDD exposure and bile flow, with maximum bile flow observed in the 25 μg/kg dose group at 16 days. Even with this increased bile flow, however, the cumulative biliary excretion of ICG was decreased in a dose-dependent manner with the greatest depression observed 7 and 16 days after the exposure to 2,3,7,8-TCDD. The levels of ICG in the plasma and liver was higher in treated animals than in control animals, while the concentration in the bile was lower, reflecting the decrease in total excretion of ICG.

Yang and Peterson (1977) compared the effect of 2,3,7,8-TCDD on the biliary excretion of the organic neutral compound, ouabain, with that of the organic anions phenol-3,6-dibromophthalein (DBSP) and sulfobromophthalein (BSP) in male Holtzman rats. Animals were intubated with 2,3,7,8-TCDD at doses of 10 or 25 μg/kg and excretion was evaluated periodically between 2-4 days postexposure. The biliary excretion of ouabain was depressed in a dose-related manner starting on the second day post-treatment, with maximum depression developing between 10 and 20 days, and some recovery observed by day 40. Decreases in bile flow followed a pattern similar to that observed for ouabain. The pattern of biliary excretion was different for DBSP and BSP in which only a transient small decrease was observed 10 days after exposure in the high dose group. In the low dose animals there was actually an increase at days 10 and 25 in the excretion of the anions. The results obtained for DBSP and BSP differ sharply from those for the organic neutral ouabain or those reported by Hwang (1973) for the organic anion ICG, in which a dose-related decrease in biliary excretion was observed. The authors concluded that the effects of 2,3,7,8-TCDD on the multiple pathways involved in biliary excretion depend on the specific compound being studied.

In the guinea pig and rhesus monkey, which do not develop significant liver pathology after exposure to 2,3,7,8-TCDD, there was also little change in ICG blood clearance rates, while in the rabbit, which develops 2,3,7,8-TCDD-induced liver damage similar to the rat, there was reduced blood clearance of ICG (Seefeld et al., 1979, 1980). In the rabbit, there were increases in serum sorbitol dehydrogenase and GPT activity as further indications of 2,3,7,8-TCDD-produced liver damage. In the monkey, which received 2,3,7,8-TCDD by gavage at doses of 5, 25 or 75 μg/kg, there was an initial slight increase in the blood clearance of ICG at 2 days post-treatment, followed in the two higher dose groups by a dramatic decrease a few days before death. Although some serum enzymes (sorbitol dehydrogenase and GPT) indicative of liver damage were elevated, the histopathology of the liver was within normal limits. It appears that major effects on biliary excretion occur only in species that are sensitive to the hepatotoxic effects of 2,3,7,8-TCDD.

Other gross signs of the hepatotoxic effects of 2,3,7,8-TCDD observed in some species included fatty degeneration and porphyria. Early observations by Cunningham and Williams (1972) described a decrease in _in vivo_ (1 hour pulse) incorporation of ^{3}H sodium acetate into liver lipids after exposure of male Wistar rats to 2,3,7,8-TCDD. The rats (12-16 animals) were treated with 2,3,7,8-TCDD at a dose of 10 μg/kg followed in either 3 or 7 days by the assessment of lipid synthesis. At 3 days incorporation decreased from 258 to 98 dpm/mg lipid in the control and treated animals, respectively. There was an approximately similar decrease observed 7 days postexposure.

When individual classes of lipids were examined, there was a decrease in the synthesis of triglycerides, diglycerides and phospholipids. Although Cunningham and Williams (1972) observed that 2,3,7,8-TCDD decreased lipid synthesis, Albro et al. (1978) reported an increase in total lipids in the livers of rats 13 days after treatment with 2,3,7,8-TCDD at a lethal dose of 50 µg/kg. For individual classes of lipids there was an increase in free fatty acids and cholesterol esters, while no change occurred in the content of phospholipids, free cholesterol or triglycerides. The fatty changes in the liver were confirmed by ultrastructural examination of liver specimens. At a sublethal dose of 10 µg/kg there was a different pattern of lipid accumulation, with triglycerides and fatty acids increased and cholesterol esters decreased. The changes in the lipid profile of the liver was attributed to 2,3,7,8-TCDD induced mobilization of body fat, a decrease in lysosomal acid lipase (74% decline in this enzyme 10 days after a 50 µg/kg dose of 2,3,7,8-TCDD) and an increase in lipid peroxidation as indicated by a sharp increase in the production of lipofuscin pigments.

Porphyria was initially characterized quantitatively in mice by Goldstein et al. (1978). Groups of 12 male C57Bl mice received 4 weekly intubations of 2,3,7,8-TCDD at doses of 0.0, 1, 5 or 25 µg/kg, or a single dose of 150 µg/kg followed 21-25 days after treatment by analysis of the liver for porphyrins. Porphyrin levels were unchanged except in the 25 and 150 µg/kg groups where the levels were increased 2000- and 4000-fold, respectively. The difference in responsiveness to the development of porphyria was studied by Smith et al. (1981) in C57Bl mice which were sensitive to, and DBA/2 mice which were insensitive to, the toxicity of 2,3,7,8-TCDD.

Male and female C57Bl mice had a dose-related increase in hepatic porphyrins in the two high dose groups 3 weeks after a single exposure to 2,3,7,8-TCDD at 0.0, 5, 15, 50 or 75 μg/kg, while only minimal nondose-related changes in hepatic porphyrin were observed in DBA/2 mice exposed to up to 1200 μg/kg. In the sensitive C57Bl mice there was only a small difference in hepatic porphyrin between the sexes even though males were >3 times as sensitive to the toxic effects of 2,3,7,8-TCDD than females (see Table V-1). Results similar to those above were reported for urinary porphyrin levels in male C57Bl and DBA/2 mice given 6 weekly doses of 2,3,7,8-TCDD at 25 μg/kg (Jones and Sweeney, 1980). In the sensitive strain, the initial elevation of porphyrin occurred in the second week.

In rats increased urinary porphyrin was observed only after subchronic exposure to 2,3,7,8-TCDD (Cantoni et al., 1981). Female CD rats were orally administered a weekly dose of 2,3,7,8-TCDD at levels of 0.01, 0.1 and 1.0 μg/kg for 45 weeks. The initial increase was observed in the high dose group at 3 months, and in the other two groups at 4 months, after the start of exposure. Not only did the absolute amount of porphyrin increase, but the relative distribution also changed to compounds containing more carboxyl groups. Only in the high dose group did the livers, at the terminal necropsy, show signs of excess porphyrin under examination by UV light.

In attempts to understand the mechanism of 2,3,7,8-TCDD induced porphyria, the effects of 2,3,7,8-TCDD on the enzymes involved in the synthesis and catabolism of porphyrin have been studied. Goldstein et al. (1978) showed that δ-aminolevulinic acid synthetase, a rate-limiting enzyme in porphyrin synthesis, was slightly increased (2-fold) in male C57Bl mice given 4 weekly doses of 2,3,7,8-TCDD at 25 μg/kg. This dose of 2,3,7,8-TCDD increased liver porphyrin levels 2000-fold. Catabolism of porphyrin by uroporphyrinogen decarboxylase (UD) also appeared to be decreased in 2,3,7,8-TCDD treated mice. Smith et al. (1981) reported a decrease in UD activity from ~25-7 nmoles/hr/g liver in male and female C57Bl mice 3 weeks after a single oral exposure to 2,3,7,8-TCDD at a dose of 75 μg/kg. No effect of 2,3,7,8-TCDD on UD activity was observed in DBA/2 mice which were insensitive to the induction of porphyria. A time course of changes in UD activity with length of time after exposure to 2,3,7,8-TCDD indicated a steady decline in activity starting 3 days after exposure to 2,3,7,8-TCDD, which continued until day 21 when the study was terminated. Sweeney and Jones (1978) reported similar results after 5 weekly doses of 2,3,7,8-TCDD at 25 μg/kg. In this study the UD activity declined ~48% in C57Bl mice and only 4% in DBA/2 mice. Other factors besides the increase in δ-aminolevulinic acid synthetase and the decrease in UD activity may also participate in the dramatic increase in liver porphyrin in mice associated with exposure to near lethal doses of 2,3,7,8-TCDD.

As a result of the protracted time observed between exposure to 2,3,7,8-TCDD and the development of toxic effects, as well as the reported teratogenic and carcinogenic potential of 2,3,7,8-TCDD, investigations have been conducted to determine the influence of 2,3,7,8-TCDD on DNA synthesis in the liver. Greig et al. (1974) measured the in vivo incorporation of ^{3}H-thymidine (1 hour pulse) into liver DNA of male and female Porten strain rats after a single exposure to 2,3,7,8-TCDD at doses of 10 and 200 μg/kg.

When the 2,3,7,8-TCDD was given either 0, 24 or 72 hours before a 3/4 partial hepatectomy there was only a slight, but not significant, decrease in thymidine incorporation observed when DNA synthesis was measured 24 hours after the operation.

Although 2,3,7,8-TCDD had no effect on in vivo DNA synthesis, similar studies by Conway and Matsumura (1975) and Dickens et al. (1981) demonstrated an increase in thymidine incorporation when determined in vitro. Conway and Matsumura (1975) administered male Sprague-Dawley rats 2,3,7,8-TCDD at a dose of 5 μg/kg followed in 10 days by removal of the liver and the in vitro determination of DNA synthesis in liver slices. Incorporation of thymidine into the nuclei increased from 29 cpm/mg in control animals to 45 cpm/mg in treated animals. A similar near doubling of DNA synthesis was observed by Dickens et al. (1981); however, when DNA synthesis was stimulated by a 1/3 partial hepatectomy, thymidine incorporation into liver slices was increased 10-fold in rats treated 5 days earlier with 2,3,7,8-TCDD as compared with hepatectomized controls. The onset of DNA synthesis after partial hepatectomy (~20 hours) was the same in both 2,3,7,8-TCDD treated and control animals; however, the treated animals had a more rapid and extensive increase in DNA synthesis between 20 and 32 hours after the partial hepatectomy. The rates of DNA synthesis were again the same in both groups 35 hours after the operation. It was shown by hydroxyurea inhibition that the DNA synthesis in both the treated and control animals was predominantly semiconservative. Further studies are needed to determine the reason for the difference observed between in vitro and in vivo measurements of DNA synthesis in the liver after exposure to 2,3,7,8-TCDD.

Extensive hepatic necrosis in the rabbit may be responsible for death in this species (Poland and Knutson, 1982).

Besides the effects on the liver of 2,3,7,8-TCDD exposure described above, it is known that 2,3,7,8-TCDD is a potent inducer of microsomal enzymes. These studies will be discussed in the Enzyme Induction by TCDD Section, which describes the ability of this xenobiotic to induce microsomal enzymes in a number of tissues and organs.

Effects on Other Organ Systems -- The most noticeable feature of 2,3,7,8-TCDD toxicity is the loss of body weight and the apparent "wasting away" until death. Since decreased food consumption may not totally account for these findings, the effect of 2,3,7,8-TCDD on intestinal absorption has been studied. Madge (1977) assessed the ability of the intestine to absorb D-glucose, D-galactose, L-arginine and L-histidine using the everted intestinal sac technique in CD-1 mice exposed to 2,3,7,8-TCDD. In measurements made 7 days after treatment with doses of 0.0, 10, 25, 75, 150, 200 or 300 μg/kg, D-glucose was absorbed to a lesser degree at all doses than in control animals. The two low doses produced a dose-related decrease in absorption; however, at doses of ≥75 μg/kg the decrease was uniform. At a dose of 150 μg/kg, decreased absorption of D-glucose was slight 3 days after treatment, became maximally decreased by 7 days, and this depressed level was maintained for 28 days, at which time the study was terminated. Providing D-mannose to the incubation mixture as an energy supply increased the absorption of D-glucose to control levels; however, the amount of D-glucose on the serosal side was still lower than control levels. This suggested that intestinal utilization of D-glucose was taking place and might account for some of the observed malabsorption. Treatment with

2,3,7,8-TCDD had no effect on the absorption of the other compounds investigated. In a similar experiment in Sprague-Dawley rats, Ball and Chhabra (1981) also observed malabsorption of D-glucose. In this study, however, absorption of leucine was also decreased. The decrease in leucine absorption took longer to manifest itself, with a significant decrease only observed 2 weeks after treatment with 2,3,7,8-TCDD.

In contrast to the results observed for D-glucose, intestinal iron transport was shown to be elevated by exposure to 2,3,7,8-TCDD. Manis and Kim (1979a) examined the effect of prior treatment of male Sprague-Dawley rats on the 30-minute transport of ^{59}Fe out of a duodenal loop created by ligating a section of the intestine in situ. At single 2,3,7,8-TCDD doses of between 22 and 84 μg/kg there was increased serosal transfer of ^{59}Fe measured 48 hours after treatment. At doses >42 μg/kg the increase was -100%. The time after treatment at which serosol transfer was greatest was 1 day, with rapid decline in stimulation to near the levels of controls observed on days 2-7. There was also an apparent effect of route of administration, with gavage treatment being more effective in inducing iron transport than i.p. injection. In similar experiments calcium transport was decreased, and galactose and proline transport were unaffected by prior exposure to 2,3,7,8-TCDD. Manis and Kim (1979b) had identical results when the everted intestinal sac was used to assess iron transport. It was interesting to note that only duodenal sacs were stimulated, with no effect of 2,3,7,8-TCDD exposure observed in the adjacent distal segment of the intestine. Increased iron transport was also observed by Manis and Kim (1979a) in an unidentified strain of mice. Increased iron transport may be one of the earliest effects of 2,3,7,8-TCDD; however, at present the toxicologic relevance of this transient disturbance in iron transport is unknown.

One of the common gross observations of 2,3,7,8-TCDD toxicity is severe edema, suggestive of a breakdown in salt and water homeostasis. These observations prompted investigations to determine the effect of 2,3,7,8-TCDD on the function of the kidney. Pegg et al. (1976) measured renal function *in vitro* using renal cortical slices obtained from male Sprague-Dawley rats 3 and 7 days after intubation with 2,3,7,8-TCDD at doses of 10 or 25 μg/kg. (These results were also described by Hook et al., 1977). Anion and cation transport were measured by the respective accumulation of p-aminohippuric acid and N-methylnicotinamide into the cortical slices. Anion accumulation was lower in the high dose group, while cation transport was lower at both dose levels tested. The decrease in anion transport was confirmed in an *in vivo* study. Ammoniogenesis and gluconeogenesis were not affected in 2,3,7,8-TCDD treated rats, even when the animals were made acidotic, indicative of no effect on the kidneys' ability to maintain acid base balance. Also, sodium reabsorption was shown *in vivo* to be within normal range. Since decreases in cation and anion transport were the only effects observed, and since these compounds are transported by a different mechanism, the authors concluded that the effects of 2,3,7,8-TCDD were merely a general decrease in kidney functon reflecting the poor condition of the treated animals (animals in all treated groups had decreased weight gain), and not a cause of debilitation.

Although kidney function was only minimally affected by exposure to 2,3,7,8-TCDD, Greig et al. (1974) demonstrated that pre-exposure to 2,3,7,8-TCDD could reduce the ability of the rat kidney to respond to stimuli of DNA sythesis. Folate-stimulated DNA synthesis measured *in vivo* in Porten strain rats was decreased between 67 and 25% in animals receiving 2,3,7,8-TCDD at a dose of 10 μg/kg on day 0-9 before administration of folic acid. No significant difference in folate-stimulated DNA synthesis was observed if 2,3,7,8-TCDD was given 23 hours after folic acid. The lack of effectiveness of administering 2,3,7,8-TCDD shortly after treatment with folic acid suggested that 2,3,7,8-TCDD did not directly interact with cellular DNA, nor inhibit the protein synthesis necessary to support folate-stimulated DNA synthesis. Similar inhibitory effects of 2,3,7,8-TCDD were observed when lead acetate was used to stimulate kidney DNA synthesis. The mechanism by which 2,3,7,8-TCDD prevents the kidney from responding to proliferative stimuli is not known, although it was demonstrated that another agent capable of inducing microsomal enzymes, 3-methylcholanthrene (3-MC), had similar effects on the kidney.

Additionally a number a hematologic and clinical chemistry changes have been observed in the blood of laboratory animals after exposure to 2,3,7,8-TCDD. Many of these changes, as described by Zinkl et al. (1973), reflect damage to previously described organ systems. In female CD rats given 30 daily doses of 2,3,7,8-TCDD at levels of 0.1, 1.0 or 10 μg/kg, the clinical chemistry of the serum reflected liver damage. In the high dose group, serum GOT and serum GPT were elevated starting 13-17 days after initial treatment. There was a marginal change in GPT in the mid-dose group and lactic dehydrogenase (LDH) in the high group, but the increases were only transitory. Serum cholesterol was increased in the high dose animals starting at day 10, with a transitory increase again observed in the mid-dose group. Conversely, there was a decrease in serum protein from day 24 on in the high-dose animals. Along with these clinical chemistry changes indicative of liver damage, the only other major effect observed in the blood was thrombocytopenia. The decrease in platelet count was detected early, by day 3, in the 10 and 1 µg/kg groups, while in the 0.1 µg/kg group a significant decrease was not observed until day 17. Thrombocytopenia was also observed in female guinea pigs after 8 weekly oral doses of 2,3,7,8-TCDD at 0.2 µg/kg, and in mice (administered a single dose of 1.0, 10 or 50 µg/kg). In guinea pigs lymphopenia was also observed. Other hematologic changes were attributed to hemoconcentration.

In a more extensive investigation of 2,3,7,8-TCDD-induced hyperlipidemia in male Sprague-Dawley rats, Poli et al. (1980) treated animals with a single i.p. injection of 2,3,7,8-TCDD at 2 doses of 2.5, 5, 10 and 20 µg/kg. At day 21 after treatment there was a dose-related increase in total plasma cholesterol and high density lipoprotein cholesterol, while no change was observed in triglycerides or very low and low density lipoproteins (VLDL and LDL, respectively). At a dose of 20 µg/kg the maximum increase in HDL cholesterol and total cholesterol occurred 30 days after treatment, and a significant elevation was still present at 60 days after treatment when the study was terminated. Slight changes in the apoprotein of HDL from 2,3,7,8-TCDD rats and control rats were indicative of new apoprotein synthesis. Although the increases in HDL cholesterol may be in response to eliminating excess lipids, the exact function has not been clearly shown. There is some evidence from studies of workers exposed to 2,3,7,8-TCDD that there were reduced levels of blood HDL cholesterol and raised total cholesterol as compared with a matched control group (Walker and Martin, 1979).

In contrast to rats, male Hartley strain guinea pigs given a single i.p. injection of 2,3,7,8-TCDD at a dose of 2 μg/kg had increased hyperlipidemia characterized by increases in VLDL and LDL (Swift et al., 1981). In animals sacrificed 7 days after exposure to 2,3,7,8-TCDD, there was an increase in total serum lipid, cholesterol esters, triglycerides and phospholipids, when comparison was made with pair-fed, weight-paired or ad libitum fed control groups. Serum-free fatty acids were not changed quantitatively; however, some qualitative changes occurred, reflecting an increase in the types of fatty acids that were abundant in the adipose tissue of guinea pigs. Anaylsis of lipoproteins revealed a 19-fold increase in VLDL and a 4-fold increase in LDL, with no change observed in the levels of HDL. The VLDL was also qualitatively different in the 2,3,7,8-TCDD treated animals, containing less cholesterol ester and an altered C apoprotein. The importance of these qualitative changes is unclear. The hyperlipidemia may result from the 2,3,7,8-TCDD mobilization of free fatty acids, which are then used in the synthesis of VLDL and are subsequently formed into LDL. The relationship of the changes in serum lipid levels to the mechanism of 2,3,7,8-TCDD toxicity needs further study.

Elovaara et al. (1977) observed some changes in biochemicals of the brain of male Wistar and heterozygous Gunn rats given a single intubation of 2,3,7,8-TCDD at a dose of 20 μg/kg. At 7 days post-treatment, there was a small but significant decrease as compared with vehicle treated control animals in both the protein and RNA content of the Wistar rats, while levels of acid proteinase and DT-diaphorase (an enzyme induced by 2,3,7,8-TCDD in the liver) had a small but significant increase in the heterozygous Gunn rats. There were no significant changes observed in homozygous rats given

2,3,7,8-TCDD at 20 μg/kg. The authors noted that acid proteinase may participate in chemically induced degeneration of the brain.

Immunological Effects -- During acute toxicity studies with 2,3,7,8-TCDD, thymic atrophy was noted as a consistent effect in all species that have been investigated. This finding suggested that 2,3,7,8-TCDD may alter the immune response, and initiated immunotoxicity studies in exposed animals. In guinea pigs treated with 8 weekly oral doses of 2,3,7,8-TCDD (0, 0.008, 0.04, 0.2 or 1.0 μg/kg bw), body weight, spleen weight and thymus weight were depressed, adrenal weight was increased and leukocyte and lymphocyte counts were elevated (Vos et al., 1973). Upon histological examination, 2,3,7,8-TCDD-exposed rats had a severe depletion of lymphocytes from the thymic cortex (Vos and Moore, 1974). Hematological changes were noted in rats exposed to 10 and 14 daily doses of 10 μg/kg 2,3,7,8-TCDD (Weissberg and Zinkl, 1973). Increased red blood cell count, decreased platelet count, increased neutrophil count and increased packed cell volumes were reported in 2,3,7,8-TCDD-exposed rats. A summary of the data available on the immunotoxic effects of 2,3,7,8-TCDD in animals is presented in Table V-4. A review of immunotoxicity and immunosuppression was reported by Vos (1977).

Vos et al. (1973) investigated the humoral and cell-mediated immune response in Hartley guinea pigs, CD rats and B6D2F$_1$ mice. The humoral immune response was tested in 2,3,7,8-TCDD-treated hamsters by injecting tetanus toxoid (subcutaneously) into the footpad and later testing for the concentration of tetanus antitoxin from the serum by an immunodiffusion technique. Cell-mediated immunity was tested by injecting *Mycobacterium*

TABLE V-4

Immunological Effects of 2,3,7,8-TCDD in Animals

Species/ Strain	Sex	Exposure Route	Dose(s)	Duration of Exposure	Minimum Effective Dose	Parameter	Effect	Reference
Mice/B6D2F$_1$	M	gavage	0, 0.2, 1.0, 5.0, 25.0 µg/kg bw/week	4 weeks	NA	bw	no change	Vos et al., 1973
					5.0 µg/kg bw/week	thymus weight	decreased	
					5.0 µg/kg bw/week	graft-versus-host response	decreased	
Mice/C57Bl/6	F,M	maternally administered (gavage)	0, 1.0, 2.0, 5.0, 25.0 µg/kg	4 or 6 weeks (3 or 5 administrations)	1.0 µg/kg bw/week	thymus weight	decreased	Vos and Moore, 1974
					25.0 µg/kg bw/week	PHA response	decreased	
					2.0 µg/kg bw/week	skin graft rejection	prolonged	
Mice/ C57Bl/6Jfh	M	gavage	0, 0.5, 1, 5, 10, 20 µg/kg bw/week	4 weeks	1.0 µg/kg bw/week	*Salmonella* infection	increased mortality and decreased time to death	Thigpen et al., 1975
Mice/Swiss	M	gavage	0, 1.5, 5, 15, 50 µg/kg bw/week	4 weeks	1.5 µg/kg bw/week	endotoxin (*E. coli*) susceptibility	increased mortality	Vos et al., 1978a
Mice/B6C3F$_1$	F	*in vitro* (spleen cells)	0.5, 5.0, 50 ng/mℓ	5-60 seconds	50 µg/mℓ	protein, DNA, and RNA synthesis	decreased	Luster et al., 1979a,b
Mice/Swiss-Webster	F,M	maternally administered (diet)	0, 1, 2.5, 5, 10, 20 ppb (dietary)	10 weeks (pre-gestation and 3 weeks post-parturition)	2.5 ppb	antigenic RBC reaction	decreased	Thomas and Hinsdill, 1979
					2.5 ppb	thymic cortex	atrophy	
					5 ppb	contact sensitivity to DNFB	decreased	
					1 ppb	endotoxin (*Salmonella*) susceptibility	increased mortality	
					NA	*Listeria* infection	no change	
Mice/CD	M	gavage	0, 0.01, 0.1, 1.0, 10.0 µg/kg bw/week	up to 8 weeks	0.01 µg/kg bw/week	serum immunoglobin level	increased	Sharma and Gehring, 1979
					1.0 µg/kg bw/week	serum immunoglobin level	decreased	
Mice/CD	M	*in vitro*	10^{-4}-10^{-8} M	single	10^{-8} M	lymphocyte blastogenic transformation	increased	Sharma and Gehring, 1979

TABLE V-4 (cont.)

Species/ Strain	Sex	Exposure Route	Dose(s)	Duration of Exposure	Minimum Effective Dose	Parameter	Effect	Reference
Mice/Swiss-Webster	F	oral (diet)	0, 10, 100 ppb	5 weeks (or more)	10 ppb	tetanus response	decreased	Hinsdill, et al., 1980
					10 ppb	antigenic RBC response	decreased	
					10 ppb	sensitization to DNFB	decreased	
					10 ppb	resistance to *Salmonella*	increased mortality	
					10 ppb	resistance in *Listeria*	increased mortality	
Mice/ C57Bl/6J	M	i.p.	0, 1, 2, 6, 30 µg/kg bw	single injection	1 µg/kg	macrophage and natural killer cell activity	no change	Mantovani et al., 1980
						macrophage and natural killer cell number	decreased	
						antibody production	decreased	
Mice/B6C3F$_1$	M,F	maternally administered	0, 1.0, 5.0, 15.0 µg/kg bw/day	4 days during gestation and lactation	1.0 µg/kg bw/day	*L. monocytogenes* susceptibility	increased	Luster et al., 1980
					1.0 µg/kg bw/day	PYB6-tumor susceptibility	increased	
					5.0 µg/kg bw/day	bone marrow hypocellularity	increased	
Mice/C57Bl/6	M	i.p.	0, 0.4, 4.0, 40 µg/kg bw/week	4 weeks	4.0 µg/kg bw/week	thymus atrophy	increased	Clark et al., 1981
					0.4 µg/kg bw/week	cytotoxic T-cell response	decreased	
Mice/C57Bl/6	M	i.p.	0, 0.004, 0.04, 0.4 µg/kg bw/week	4 weeks	0.004 µg/kg bw/week	*in vitro*-generation of cytotoxic T-cells	decreased	Clark et al., 1981
Rat/CD	F	oral	0, 0.2, 1.0, 5.0 µg/kg bw/week	6 weeks	5.0 µg/kg bw/week	bw	decreased	Vos et al., 1973
					5.0 µg/kg bw/week	thymus weight	decreased	
					NA	tuberculin hypersensitivity	no change	
Rat/CD	F	oral	0, 10 µg/kg bw/day	10, 14 days	10 µg/kg bw/day	erythrocyte count	increased	Weissberg and Zinkl, 1973
					10 µg/kg bw/day	platelet count	decreased	
					10 µg/kg bw/day	neutrophil count	increased	

TABLE V-4 (cont.)

Species/ Strain	Sex	Exposure Route	Dose(s)	Duration of Exposure	Minimum Effective Dose	Parameter	Effect	Reference
Rat/F-344	F,M	maternally administered	0, 1.0, 5.0 μg/kg bw/dose	4 or 6 weeks (3 or 5 administrations)	1.0 μg/kg bw/dose	bw and thymus weight	decreased	Vos and Moore, 1974
					5.0 μg/kg bw/dose	spleen weight	decreased	
					5.0 μg/kg bw/dose	PHA response	decreased	
					5.0 μg/kg bw/dose	graft-versus-host response	decreased	
					5.0 μg/kg bw/dose	skin graft rejection	prolonged	
					NA	pseudorabies virus infection	no change	
Rat/Fischer	F,M	maternally administered (NR)	NR	4-6 weeks (during gestation and neonatally)	NR	Con A and PHA response	decreased	Moore and Faith, 1976
					NR	oxazolone skin hypersensitivity	decreased	
Rat/Fischer-Wistar	F,M	maternally administered (NR)	0, 5 μg/kg bw/dose	3 or 4 applications during gestation and neonatally	5 μg/kg bw/dose	antibody production to bovine gamma globulin	no effect	Faith and Luster, 1979
					5 μg/kg bw/dose	PHA and Con A response	decreased	
					5 μg/kg bw/dose	thymus and bw	decreased until 135 days	
Rat/Sprague-Dawley	M	i.v.	0, 1 μg/kg bw	single injection	1 μg/kg bw	thymic RNA synthesis	decreased	Kurl et al., 1982
						thymic RNA polymerase activity	decreased	
Guinea pig/ Hartley	F	gavage	0, 0.008, 0.04, 0.2, 1.0 μg/kg bw	8 weeks	0.04 μg/kg bw/week	bw	decreased	Vos et al., 1973
					0.04 μg/kg bw/week	thymus weight	decreased	
					0.04 μg/kg bw/week	tuberculin hypersensitivity	decreased	
					0.2 μg/kg bw/week	tetanus antitoxin	decreased	

M = male; F = female; i.p. = intraperitoneal i.v. = intravenous; PHA = Phytohemagglutinin; Con A = Concanavalin A; RBC = red blood cell; DNFB = 2,4-dinitro, 1-fluorobenzene; NA = Not applicable; NR = Not reported *tuberculosis* (subcutaneously) into guinea pigs on day 35 of 2,3,7,8-TCDD treatment (during a schedule of 8 weekly doses). Intradermal tuberculin hypersensitivity was determined by measurements of skin thickening on days 47 and 54. Decreased skin hypersensitivity was noted in hamsters treated with 0.04 g 2,3,7,8-TCDD/kg and higher doses. Decreased tetanus antitoxin levels were evident in guinea pigs treated with 0.2 µg 2,3,7,8-TCDD/kg, but not at lower dose levels. Vos et al. (1973) also tested the cell-mediated immunity in rats exposed to 2,3,7,8-TCDD (0, 0.2, 1.0 or 5.0 µg/kg, once weekly for 6 weeks). *M. tuberculosis* was injected into rats by day 28 of the treatment period, followed by intradermal hypersensitivity testing on day 42. No changes in the thickness of skin were noted in 2,3,7,8-TCDD-treated rats when compared with controls.

Mice were used to test the effect of 2,3,7,8-TCDD on cell-mediated immunity by use of the "graft-versus-host" experiment (Vos et al., 1973). In this test, spleen cells from 2,3,7,8-TCDD-exposed mice (0, 0.2, 1.0 or 5.0 µg/kg once weekly for 4 weeks) of the C57Bl/6 strain were injected into the right footpad of a hybrid recipient mouse (C57Bl/6 x DBA-2). Donor cells possessing sufficient activity will respond to the DBA-2 antigen on the host cells, resulting in the enlargement of the popliteal lymph node. Host cells are tolerant of the donor cells since both have C57Bl/6 antigens. In this test Vos et al. (1973) noted a significant ($p<0.01$) dose-related decrease in the activity of 2,3,7,8-TCDD-treated spleen cells (as measured by the degree of popliteal lymph node enlargement on the site of the spleen cell injection). Lymph node enlargement was significantly less ($p<0.01$) in hybrid recipient mice receiving spleen cells from mice treated with 5 µg 2,3,7,8-TCDD/kg/week than from donor cells of untreated mice.

Studies continued in an attempt to identify the mechanism of 2,3,7,8-TCDD-induced immunodeficiency. Rats (F-344) exposed pre- and postnatally by maternal dosing (1 or 5 μg 2,3,7,8-TCDD/kg administered to dams on days 11 and 18 of gestation and 0, 7 and 14 postnatally) had prolonged times until graft rejection, decreased spleen cell graft-versus-host activity and decreased binding response to phytohemagglutinin (PHA) (Vos and Moore, 1974; Moore and Vos, 1974). Response to conconavalin A (Con A), a humoral immune response, was actually increased.

Since thymus-derived lymphocytes (T-cells) play a central role in cell-mediated immunity and host defense mechanisms, interest turned to these areas of immunology. The effect of 2,3,7,8-TCDD on host resistance to infection, a vital measure of immune response, was tested by Thigpen et al. (1975) in male pathogen-free mice (C57Bl/6Jfh). 2,3,7,8-TCDD was administered to mice at 0.5, 1, 5, 10 or 20 μg/kg once weekly for 4 weeks followed by inoculation with Salmonella bern 2 days after the final 2,3,7,8-TCDD administration. Mortality rates and "time until infection" were used to determine the immunological effect of 2,3,7,8-TCDD. A significant ($p<0.05$) increase in mortality and decrease in time of infection were noted in groups treated with 1 μg/kg or higher doses of 2,3,7,8-TCDD when compared with controls. 2,3,7,8-TCDD at 0.5 μg/kg did not alter these parameters and was regarded as a no effect level. The immune-resistance of mice to S. bern is therefore reduced by treatment with 1 μg 2,3,7,8-TCDD/kg/week (for 4 weeks).

Pretreatment with 2,3,7,8-TCDD greatly enhances the susceptibility of mice to *E. coli* endotoxin (Vos et al., 1978a). Injection of 250 μg of endotoxin to mice pretreated with 0, 1.5, 5 and 15 μg 2,3,7,8-TCDD/kg resulted in 0/5, 1/5, 6/6 and 6/6 deaths, respectively. Mice pretreated with 15 and 50 μg 2,3,7,8-TCDD/kg and injected with 10 μg of endotoxin had 1/4 and 2/4 deaths, respectively. Mice treated with lower doses of 2,3,7,8-TCDD were not susceptible to this quantity of endotoxin. Increased mortality (2/6) in a control group was noted only when 500 μg of endotoxin was administered, while 10 μg of endotoxin was sufficient to cause similar mortality (2/5) in mice treated with 50 μg 2,3,7,8-TCDD/kg.

The immunocompetence of 5-week-old offspring of Swiss-Webster mice fed diets containing 1, 2.5, 5, 10 or 20 ppb 2,3,7,8-TCDD was tested by several means (Thomas and Hinsdill, 1979). The number of cells reactive to antigenic RBC, differential white blood cell counts, organ weights, histopathologies, hypersensitivity to 2,4-dinitro-1-fluorobenzene (DNFB) and the resistance to *E. coli* lipopolysaccharide (LPS), *Listeria monocytogenes* and *Salmonella typhimurium* LPS were all measured for mice exposed to different levels of 2,3,7,8-TCDD. Adult female mice were exposed to 2,3,7,8-TCDD for 4 weeks before mating, throughout gestation and for 3 weeks postparturition. Young mice being tested for immunotoxicity were therefore exposed to 2,3,7,8-TCDD only *in utero* and through lactation. The typical decrease in thymus weight was noted in mice exposed to 2.5 and 5.0 ppb but was not evident in the 1.0 ppb group. A decrease in the number of plaque-forming cells (PFC) reactive to sheep RBCs was significantly reduced in the 2.5 and 5.0 ppb 2,3,7,8-TCDD-exposed groups. (Because of the poor survival of young in the 10 and 20 ppb 2,3,7,8-TCDD-exposed groups, results and comparisons were usually reported for the three lower dose groups). The humoral content of anti-RCD antibodies, however, was not lower in 2,3,7,8-TCDD-exposed groups when compared with controls. A decrease in the skin hypersensitivity to DNFB following sensitization was noted in all 2,3,7,8-TCDD-treated groups (only the 5 ppb group was statistically reduced from controls). 2,3,7,8-TCDD caused an increased susceptibility (increased mortality level) to _S. typhimurium_ in a dose-related fashion. The response to _E. coli_ LPS and _L. monocytogenes_ was not different from controls. 2,3,7,8-TCDD exposure did not alter the response of lymphocytes (Band T-cells) _in vitro_ to Con A, nor was mitogen-induced lymphocyte proliferation affected (Thomas and Hinsdill, 1979).

Similar findings were reported in Fischer/Wistar rats exposed to 2,3,7,8-TCDD during gestation (18th day) and neonatally, or neonatally alone (on days 0, 7 and 14) (Faith and Luster, 1979). Dams were treated with 5 g/kg 2,3,7,8-TCDD on each dose day. Typically, body weight and thymic weights were decreased in progeny, which lasted until 135 days of age. The thymic- and splenic-cell response to PHA and Con A was decreased in all 2,3,7,8-TCDD-treated animals and did not return to normal until day 270. Delayed hypersensitive reaction was also suppressed until 270 days of age. The production of antibodies to bovine gamma globulin, which requires T-helper cell function, was not affected by 2,3,7,8-TCDD exposure during rat development (Faith and Luster, 1979).

Neonatal $B6C3F_1$ mice, exposed to prenatal (maternal dosing on day 14 of gestation) and postnatal (days 1, 7 and 14 after birth) doses of 0, 1.0, 5.0 or 15.0 μg/kg 2,3,7,8-TCDD, were studied for immunotoxic effects and host susceptibility (Luster et al., 1980). At the 15.0 μg 2,3,7,8-TCDD/kg dose level, 70% of the neonates died with overt toxic effects (decreased body weight, liver weight, spleen weight and thymus weight). Bone marrow hypocellularity and depressed macrophages-granulocyte progenitor cells and pleuripotent stem cells were associated with 2,3,7,8-TCDD exposure at the 5.0 and 15.0 μg/kg dose levels. Hematological changes, such as decreased RBC count, hematocrit and hemoglobin, and lymphocyte count showed a dose-related response. Host susceptibility to *L. monocytogenes* and PYB6-tumor cells was tested in the 2,3,7,8-TCDD-exposed neonates. Death occurred in 73 and 40% of the *L. monocytogenes* inoculated ($1.2\text{x}10^{6}$ viable organisms) mice in the 5.0 and 1.0 μg/kg dose groups, respectively, compared with 28% of controls. Tumor development occurred in 44, 60 and 22% of the neonates inoculated with $5\text{x}10^{4}$ tumor cells from the 5.0 μg 2,3,7,8-TCDD/kg, 1.0 μg 2,3,7,8-TCDD/kg and control groups, respectively.

Hinsdill et al. (1980) reported that 2,3,7,8-TCDD administered in the diet of Swiss-Webster mice at 100 ppb for 5 weeks caused a marked suppression of total serum protein, gamma globulin and albumin, but an increase in B-globulins. At 10 ppb in the diet, 2,3,7,8-TCDD caused decreased immune response to tetanus toxoid, sheep RBC, *S. typhimurium* and *L. monocytogenes*, and lowered contact sensitivity to DNFB. This study also suggested that although young animals are more susceptible to 2,3,7,8-TCDD, older animals are still immunosuppressed and exposure *in utero* and neonatally is not more crucial than in other periods. Vos and Moore (1974) had previously reported that 1-month-old mice were more sensitive to 2,3,7,8-TCDD than were 4-month-old mice (C57Bl/6). Decreased body weight and thymus weight and spleen cell response to PHA were evident at lower doses in 1-month-old mice than in 4-month-old mice.

The effect of single i.p. doses of 2,3,7,8-TCDD (1, 2, 6 and 30 μg/kg) on peritoneal macrophage and splenic natural killer cell function in mice (C57Bl/6J) was studied by Mantovani et al. (1980) and Vecchi et al. (1980). 2,3,7,8-TCDD treatment at all dose levels did not decrease the cytostatic and cytocidal activity of macrophages or natural killer cells on a per cell basis. The total number of macrophages and splenic natural killer cells recovered from 2,3,7,8-TCDD-treated animals, however, was reduced when compared with untreated controls. Marked hypocellularity noted in the bone marrow of 2,3,7,8-TCDD-treated mice may account for the decrease in peripheral cell counts (McConnell et al., 1978b). The lack of macrophages and natural killer cells was suggested as being instrumental in the decreased resistance to infection common to 2,3,7,8-TCDD-exposed animals (Mantovani et al., 1980). Although 2,3,7,8-TCDD was a strong immunosuppressant, animals given a lethal dose of 2,3,7,8-TCDD did not appear to die from infections, nor did a germ-free environment protect them from death (Greig et al., 1973).

The actual mechanism of 2,3,7,8-TCDD immunotoxicity is unknown but several investigators have tested various hypotheses. Vos et al. (1973, 1978a,b) attempted to address the indirect causes for decreased thymic growth and altered T-lymphocyte activity following 2,3,7,8-TCDD treatment.

Vos et al. (1973) measured serum cortisol and corticosteron levels in guinea pigs exposed to 2,3,7,8-TCDD to evaluate the possible indirect immunosuppression by these hormones. There was, however, no significant difference in the level of these hormones between treated and control animals. Indirect immunosuppression of this type was unlikely. Later studies (Vos et al., 1978a,b) investigated the role of thymic hormones (thymosin) on the atrophy of the thymus during 2,3,7,8-TCDD treatment. Thymosin administered in conjunction with 2,3,7,8-TCDD did not protect mice from the typical 2,3,7,8-TCDD-induced immunotoxic alterations. Thymus weight was maintained but not increased by thymosin, and thymus-derived cells continued to show decreased responsiveness to mitogens (PHA, Con A). Thus, it is unlikely that 2,3,7,8-TCDD affects the supply or synthesis of thymic hormones which could lead to the observed immunosuppression.

van Logten et al. (1980) investigated the possible influence of the adrenal gland, hypophysis and pituitary, and growth hormone on thymic atrophy and immunosuppression following 2,3,7,8-TCDD exposure in female F-344 rats. Adrenalectomy and exogenous growth hormone had no preventative action on thymic involution. Hypophysectomized rats showed advanced thymic atrophy.

Sharma and Gehring (1979) noted that 2,3,7,8-TCDD caused stimulation of lymphocyte transformation to blast form cells (mitotically active precursors) when no mitogens were present in the culture system. This represents a phenomenon similar to actual antigenic challenge. At low doses (0.01 and 0.1 μg 2,3,7,8-TCDD/kg/week for up to 8 weeks), serum immunoglobulin levels were elevated in male CD-1 mice. Larger doses of 2,3,7,8-TCDD (1.0

and 10 μg/kg/week) resulted in a decrease in the serum immunoglobulin level. It was suggested that 2,3,7,8-TCDD may elicit an antigenic response either by combining with a body protein or by causing cellular or biochemical damage that releases antigenic proteins. Sharma and Gehring (1979) also noted that thymic atrophy was observed after 2 and 4 weeks of treatment but not after 8 weeks. There may be a recovery of thymic tissue, either by immune tolerance or immune unresponsiveness as a sort of adaptation to 2,3,7,8-TCDD-exposure and its possible antigenic complex.

Luster et al. (1979a,b) reported that 2,3,7,8-TCDD affects the immune system directly by altering lymphocyte function. The function of T-helper cells was not altered, since no change in response to bovine gamma globulin (requires T-helper cell cooperation) was noted in Wistar/Fischer and Fischer rats exposed to 2,3,7,8-TCDD. *In vitro*, 2,3,7,8-TCDD (100 ng/mℓ) suppressed DNA, RNA and protein synthesis in splenic lymphoid cells from B6C3F$_1$ (Luster et al., 1979a). 2,3,7,8-TCDD, however, did not decrease the binding of ^{3}H-Con A to lymphocytes, indicating that these receptors are not blocked by 2,3,7,8-TCDD. T-lymphocytes were more susceptible to 2,3,7,8-TCDD, measured by specific mitogen binding assays, than B-lymphocytes. These authors (Luster et al., 1979a) suggested that 2,3,7,8-TCDD may bind directly to the lymphocyte cell membrane and alter its function. Faith and Luster (1979) reported that lymphocytes from the spleen, thymus, bone marrow and lymph nodes of Fischer rats exposed to 2,3,7,8-TCDD showed abnormal homing patterns within the body. 2,3,7,8-TCDD exposure apparently altered the cell surface markers so that spleen lymphocytes were taken up by the thymus of recipient rats. These authors (Faith and Luster, 1979) suggested that 2,3,7,8-TCDD may change cellular metabolism, which alters the cell membrane constituents or may insert directly into the membrane. Kurl et al. (1982) reported that 2,3,7,8-TCDD causes changes in thymic transcription and RNA synthesis that may lead to cell surface changes. Cell surface changes could presumably result in altered antigen recognition and cell-to-cell recognition, causing immunosuppression and thymic atrophy.

Clark et al. (1981) reported that 2,3,7,8-TCDD treatment (0.4, 4.0, 40 μg/kg weekly for 4 weeks by i.p. injection) caused functional impairment of cytotoxic T-cells in C57Bl/6 male mice. The authors felt that this response was particularly sensitive to 2,3,7,8-TCDD treatment and hypothesized that 2,3,7,8-TCDD directly inhibits the function of these cells. Contrary to the hypothesis tested by these authors and that held by Luster et al. (1979a,b), 2,3,7,8-TCDD treatment impaired the generation of cytotoxic T-cells by the spleen (at doses as low as 0.004 μg/kg when detected in vitro) but did not appear directly toxic to the cytotoxic T-cells. At present, the mechanism of immunosuppression caused by 2,3,7,8-TCDD is unknown and the theories available are speculative. In a later study, however, Clark et al. (1983) reported that a 10- to 100-fold greater dose of 2,3,7,8-TCDD was required to suppress cytotoxic T-cells in DBA/2 mice as compared with C56Bl/6 mice. This indicates that susceptibility to 2,3,7,8-TCDD immunotoxicity segregates with the Ah locus which is consistent with a receptor mediated mechanism. The receptor mediated mechanism was further supported by the susceptibility of the C57Bl/6 x DBA/2J hybrid mouse to 2,3,7,8-TCDD suppression of the cytotoxic T-cells which is again consistent with the dominant inheritance of Ah (Nagarkatti et al., 1984).

Few reports are available in which the immunological effects of 2,3,7,8-TCDD exposure were studied in humans. Reggiani (1980) reported that the immunocapability of 17 people, ranging in age from 3-60 years, who had been exposed to 2,3,7,8-TCDD, was normal in all cases. In a survey of 41 workers exposed to 2,3,7,8-TCDD, Ward (1982) measured immunoglobin G, A, M, D and E, as well as lymphocytes, T-cells, B-cells, PHA response and blood cell counts. These determinations were made 10 years after workers had developed 2,3,7,8-TCDD-induced chloracne. In this group of workers, there was a significant increase in the proportion of cases with reduced IgD and IgM. It was suggested that the 2,3,7,8-TCDD-exposed group had a reduced immune capability and a deficiency in T-cell and B-cell cooperation. The immunotoxicity of 2,3,7,8-TCDD in humans cannot be properly assessed because of the paucity of data recorded soon after exposure. The most prominent effects in animals (i.e., humoral responses) were not measured in humans.

Enzyme Induction by TCDD --

In Cell Cultures. Although 2,3,7,8-TCDD has a very low toxicity to cells in culture (Beatty et al., 1975; Bradlaw et al., 1976; Knutson and Poland, 1980; Yang et al., 1983), it is an extremely potent enzyme inducer in these systems (Kouri et al., 1974; Niwa et al., 1975; Bradlaw et al., 1976; Malik and Owens, 1977; Malik et al., 1979; Bradlaw et al., 1980). This enzyme induction is so sensitive that it has been proposed as a bioassay for detecting planar polychlorinated organic compounds (Bradlaw et al., 1975, Bradlaw and Casterline, 1979; Niwa et al., 1975).

Kouri et al. (1974) found that 2,3,7,8-TCDD induced aromatic hydrocarbon hydroxylase (AHH) activity in cultured human lymphocytes to the same extent as 3-MC; however, the concentration of 2,3,7,8-TCDD necessary for maximal enzyme induction was 40-60 times less than that of 3-MC. Niwa et al. (1975) compared AHH induction by 2,3,7,8-TCDD among cell cultures (H-4-II-E, VERO, HTC, LB82, MA, Hepa-1, TRL2, ERL-2, NRKE and Chang). ED_{50} values ranged from 0.12 nM in the Hepa-1 cell line to >100 nM in the VERO and HTC cell lines. 2,3,7,8-TCDD did not induce AHH activity in LB82 cells. The responsiveness of AHH induction to 2,3,7,8-TCDD was 250-900 times greater than to 3-MC. In addition, cell cultures derived from C57Bl/6N mice were 16 times as sensitive to 2,3,7,8-TCDD as cell cultures derived from DBA/2N mice. The responsiveness of cell cultures to enzyme induction by 2,3,7,8-TCDD is thus similar to the effects seen _in vivo_. The inductive effect of 2,3,7,8-TCDD was blocked by actinomycin _D_ and cycloheximide, implying that induction involved the sythesis of new mRNA and protein. Enzyme induction by 2,3,7,8-TCDD, therefore, involves an initial RNA synthesis and continuous protein synthesis (Malik and Owens, 1977; Malik et al., 1979).

In all of these studies, there was no correlation between cytotoxicity and enzyme induction. This implies that, despite the correlation _in vivo_, there may be no direct connection betweeen enzyme induction and the toxicity of 2,3,7,8-TCDD.

In Mice and Rats. The effects of 2,3,7,8-TCDD on enzyme activity in rats and mice have been investigated extensively. 2,3,7,8-TCDD has been found to alter many enzyme activities in a wide variety of organ systems (_vide infra_). This alteration primarily results in increased enzyme activity, although 2,3,7,8-TCDD has been observed to inhibit some enzymes.

Hook et al. (1975a) reported that 2,3,7,8-TCDD supressed hepatic microsomal N-demethylation in male, but not female, rats; however, cytochrome P-450 and benzpyrene hydroxylase activity were increased. The suppression of N-demethylase activity was undetectable for 73 days following a single oral dose of 25 μg 2,3,7,8-TCDD/kg bw. The suppression of N-demethylase activity was seen only in adult animals. In 10-day-old rats, 2,3,7,8-TCDD had an inductive effect on this activity.

The inductive effects of 2,3,7,8-TCDD have been demonstrated to be organ specific. Aitio and Parkki (1978) investigated the effects of 2,3,7,8-TCDD on the activities of AHH, ethoxycoumarin deethylase, cytochrome C reductase, epoxide hydratase, UDP glucuronosyltransferase, and glutathione S-transferase in the liver, kidney, lung, small intestine and testes of male Wistar rats. Monooxygenase activity was stimulated in the liver, lung and kidney, but not in any other tissue investigated. UDP glucuronosyltransferase activity increased by a factor of 7 in the liver, by a factor of <2 in the kidney, and not at all in any other tissue. Epoxide hydratase and glutathione S-transferase activities were not affected in any of the tissues studied, although stimulation of hepatic glutathione S-transferase has been reported by other investigators (Manis and Apap, 1979). Enzyme induction has also been reported in rat mammary gland (Rikans et al., 1979), mouse testes (Mattison and Thorgeirsson, 1978), and rat prostate gland (Lee and Suzuki, 1980), but the rat adrenal gland is apparently insensitive to inductive effects of 2,3,7,8-TCDD (Guenthner et al., 1979).

In the liver of rats and mice, 2,3,7,8-TCDD affects a wide range of enzymatic activities, including DT-diaphorase (Beatty and Neal, 1976a,b), bilirubin catabolism (Kapitulnik and Ostrow, 1978), ornithine decarboxylase (Potter et al., 1982), 7-ethoxycoumarin O-demethylase (Greenlee and Poland, 1978), glutathione S-transferase (Baars et al., 1978; Manis and Apap, 1979), aldehyde dehydrogenase (Lindahl et al., 1978; Deitrich et al., 1977), uroporphyrinogen decarboxylase (Jones and Sweeney, 1977), δ-aminolevulinic acid synthetase (Goldstein et al., 1982a; Woods, 1973), UDP-glucuronosyl transferase (Marselos et al., 1978) and a number of microsomal oxidative enzyme systems (vide infra).

2,3,7,8-TCDD is four orders of magnitude more potent than 3-MC as an inducer of hepatic AHH activity; however, the dose-response curve for the two compounds are parallel and both produce the same maximal response (Poland and Glover, 1974). Simultaneous administrations of maximally inducing doses of both compounds produced no greater response than either alone and both produced a cytochrome with a shift in the absorption maximum of the carbon monoxide difference spectrum from 450 to 448 nm. In a number of studies, increased AHH activity and cytochrome P-448 synthesis have been separated (Chhabra et al., 1976); however, other researchers report an apparent connection between cytochrome P-448 and AHH induction (Kitchin and Woods, 1977, 1978a,b). Thus, 2,3,7,8-TCDD not only stimulates AHH activity by inducing cytochrome P-450 formation, but may enhance AHH activity by other mechanisms as well.

In Rabbit. The response of the rabbit is quite different from that observed in rats and mice (Hook et al., 1975a). The only changes in hepatic enzyme activities observed were suppression of benzpyrene hydroxylase and benzphetamine N-demethylase. In the same study, biphenyl 4-hydroxylase was induced in the lung and benzpyrene hydroxylase was induced in the kidney. In a similar study, a hepatotoxic dose of 2,3,7,8-TCDD (30 μg/kg) failed to alter prostaglandin synthetase activity in hepatic or renal tissue (Kohli and Goldstein, 1981).

In a series of studies, Johnson and Muller-Eberhard (1977a,b,c,d), Johnson et al. (1979), Norman et al. (1978), Liem et al. (1980) and Dees et al. (1982) isolated a series of cytochromes P-450 from rabbit liver microsomes. These cytochromes were immunologically distinct, functioned in different catalytic pathways, and responded differently to induction by polycyclic aromatic hydrocarbons. 2,3,7,8-TCDD was found to induce two cytochromes, designated as form 4 and form 6. Form 4 is the major cytochrome induced in adult rabbit liver by 2,3,7,8-TCDD; however, form 6 is the major cytochrome induced in newborn rabbit liver (Norman et al., 1978b), adult rabbit lung, and adult rabbit kidney (Liem et al., 1980; Dees et al., 1982).

Other Species. The guinea pig, the species most sensitive to the toxic effects of 2,3,7,8-TCDD, is similar to the rabbit in its response to 2,3,7,8-TCDD. Biphenyl 4-hydroxylase was induced in the liver, lung and kidney, biphenyl 2-hydroxylase was suppressed in the liver, and benzpyrene hydroxylase was induced in the kidney (Hook et al., 1975b). Testicular microsomal cytochrome P-450 content was depressed following a single oral dose of 1 μg/kg, reaching 52% of controls by 1 day and remaining at this level for 9 days (Tofilon et al., 1980). Testicular microsomal heme levels and δ-aminolevulinic acid synthetase activity were unaffected by this treatment. In contrast to the rat, 2,3,7,8-TCDD did not induce DT-diaphorase in brain, spleen, kidney, lung, heart or liver of male guinea pigs (Beatty and Neal, 1978).

Aryl hydrocarbon hydroxylase and δ-aminolevulinic acid synthetase in the chick embryo have been reported to be extremely sensitive to the inductive effects of 2,3,7,8-TCDD (Poland and Glover, 1973a,b), with maximal induction occurring with 155 pmoles/egg. This induction is relatively long lasting, with 70% of the maximum induced activity present 5 days following a single dose of 2,3,7,8-TCDD. Structure-activity studies demonstrated a perfect correspondence between the toxicity and induction potency of a series of dibenzo-p-dioxin congeners (Poland and Glover, 1973a).

Subchronic Toxicity. Four laboratory studies described the systemic toxic effects of subchronic exposure to 2,3,7,8-TCDD in rodents. Also, one semi-controlled study evaluated the toxic effects to rabbits after confinement to an area containing soil contaminated with 2,3,7,8-TCDD. No information was found in the literature searched on the effects of subchronic exposure to 1,2,3,7,8-PeCDD, and only one preliminary study was available describing the effects of subchronic exposure to a mixture of two HxCDDs in rats and mice.

Kociba et al. (1976) exposed Sprague-Dawley rats to 2,3,7,8-TCDD for 13 weeks. The animals in groups of 12 males and 12 females received the compound suspended in acetone-corn oil (1:9) by gavage 5 days/week at doses of

0.0, 0.001, 0.01, 0.1 or 1.0 µg/kg bw. At the end of the treatment period 5 rats of each sex were killed for histopathologic examination, and the remaining animals were continued for postexposure observation. This report on gross, hematologic, clinical chemistry and histopathologic (on animals terminated at the interim kill or killed when moribund) observations was prepared on data available 13 weeks after termination of treatment. Signs of toxicity were observed only at the two higher dose levels, and female rats appeared more sensitive to the toxic effects of 2,3,7,8-TCDD. During the study there were five treatment-related deaths in the high dose group females, with three occurring during treatment and two in the post-treatment period. In male animals only two deaths occurred in the post-treatment period in the high dose group. Both the male and female rats of the 0.1 and 1.0 µg/kg groups had depressed body weight; however, greater relative depression of body weight was observed in the high dose females. Other changes such as increases in bilirubin concentrations, urinary coproporphyrin excretion, and changes in relative thymus or liver-to-body weight ratio occurred in the two high-dose female groups, but only in the 1.0 µg/kg male group. Although male rats had significantly decreased hematologic values (packed cell volume, RBC count and hemoglobin) in the two high-dose groups, and these values were normal in all female rats, the authors pointed out that these results may have been an artifact resulting from dehydration-induced hemoconcentration in the female rats. No specific data were provided, however, to support this last conclusion.

After necropsy, gross examination revealed subcutaneous edema, a decrease in the size of testes and uteri, and a decrease in the number of corpora lutea. Histologic examination revealed involution of the thymus, decreased number of thymocytes, and focal necrosis and pigment accumulation in the liver. These observations were made only in the animals of the high-dose group, with the exception of a slight decrease in the number of thymocytes and mild microscopic distortion of the architecture of the liver in the group fed 0.1 μg/kg. Although histologic evidence from animals killed during the interim sacrifice was consistent with the liver and thymus being the primary target organs, in an animal that died during the study there were signs of aortic thrombosis and adrenal hemorrhage, and in a second animal there was severe anemia, suggesting possible involvement of the hematopoietic system near the time of death.

Liver toxicity was the only effect of treatment observed during histologic examination of rats (Osborne-Mendel) and mice (B6C3F_1) administered 2,3,7,8-TCDD for 13 weeks in a preliminary subchronic toxicity study designed to define an acceptable dose for a chronic toxicity study (NTP 1980a). The animals in groups of 10 males and 10 females were administered the compound in corn oil-acetone (9:1) twice a week at doses for rats of 0.0, 0.5, 1, 2, 4 and 8 μg/kg/week, and for mice at doses of 0.0, 1, 2, 5, 10 and 20 μg/kg/week. Deaths occurred at the two high-dose levels in rats, with 4 females in the 8 μg/kg/week and 1 in the 4 μg/kg/week group dying, while only 2 male rats in the 4 μg/kg/week group died. Deaths were accompanied by severe toxic hepatitis. Hepatic lesions were observed in all other rats examined in groups administered 1-8 μg/kg/week; however, not all animals in each group were submitted to necropsy. Normal liver histology was observed in the 2 male rats examined from the low-dose groups and only threshold toxic effects occurred in the low-dose female rats.

Similar effects of treatment were observed in mice, with a single death occurring in each sex at the high-exposure level, along with reports of hepatic lesions on histologic examination. In contrast to rats, female mice were less sensitive to the hepatotoxic effect of 2,3,7,8-TCDD than were the male mice. Hepatic lesions were observed in all dose groups of male mice, while the 1 and 2 μg/kg/week dose groups of female mice had normal livers. Although the group sizes were small, making conclusions tenuous, it appeared that sex differences in the sensitivity to the toxic effects of 2,3,7,8-TCDD occurred, and that the more sensitive sex may vary with species tested.

In a more extensive subchronic study in rats, King and Roesler (1974) followed the development of toxicity by a series of interim sacrifices during 28 weeks of exposure to 2,3,7,8-TCDD and a 12-week post-treatment recovery period. Groups of 35 male and 35 female Sprague-Dawley rats were intubated twice weekly with 2,3,7,8-TCDD in corn oil-acetone (9:1) at cumulative doses of 0.0, 0.1 and 1 μg/kg/week. No treatment-related deaths occurred; however, 3 animals from each group of each sex were killed after 2, 4, 8 and 16 weeks, and 10 animals of each sex were killed after 28 weeks of treatment. In addition, 3 rats of each sex were killed 4 and 12 weeks after termination of exposure. Animals were monitored for gross changes during the study and were examined for gross and histologic changes at necropsy.

Besides a dose-related decrease in body weight gain in male rats and a decrease in body weight gain in the high-dose female rats, the only effect of exposure to 2,3,7,8-TCDD was histologic changes in the liver. Liver pathology was normal in all treated groups up through the interim kill at 16 weeks. Fatty changes in the liver were considered the most important observation. The fatty changes ranged from single large lipid droplets in a few centrilobular hepatocytes to lipid droplets in all centrilobular hepatocytes with extension into the midzonal hepatocytes. No clear dose-response pattern was observed in this study; however, it did appear that the severity of fatty changes was greater in male rats. During the recovery period, fatty changes progressively decreased in severity, but were still present in some treated animals 12 weeks after cessation of exposure. Other histologic changes observed in the liver predominantly in the animals killed at 28 weeks included necrosis, increased nuclear size, subtle distortion of liver architecture, and hyperchromatic nuclei. All of these lesions were considered to be slight or mild, and less toxicologically relevant than the fatty changes. The data suggested that the liver was the most sensitive organ to the toxic effect of 2,3,7,8-TCDD, and although recovery occurred after termination of treatment, the recovery process was slow.

The recovery time was also demonstrated to be long in a subchronic study by Goldstein et al. (1982b) of 2,3,7,8-TCDD induced porphyria. Groups of 8 female Sprague-Dawley rats were given 2,3,7,8-TCDD in corn oil-acetone (7:1) weekly by gavage for 16 weeks at doses of 0.0, 0.01, 0.1 or 10.0 μg/kg/week and killed 1 week after the last treatment. Additional groups of rats received doses of 0.0 or 1.0 μg/kg/week for 16 weeks and were allowed to recover for 6 months. The high-dose level was lethal to all animals within 12 weeks, while the only other gross sign of toxicity was a decrease in body weight gain in the group receiving 1.0 μg/kg/week. After 16 weeks of exposure to 2,3,7,8-TCDD, liver porphyrins were elevated ~1000-fold in 7 of 8 animals receiving 1.0 μg/kg/week, but only 1 of 8 animals in the 0.1

µg/kg/week group had elevated porphyrin levels. No effect was observed in the low-dose animals. After a 6-month recovery period the porphyrin level in animals exposed to 1 µg/kg/week was still 100-fold higher than values in the control group. A similar pattern was observed for urinary excretion of uroporphyrin. The rate limiting enzyme in heme synthesis, δ-aminolevulinic acid synthetase, was also elevated at both the time of termination of treatment and at the end of the recovery period; however, other enzymes that were increased after 10 weeks of treatment, cytochrome P-450, AHH and glucuronyl transferase, returned to near normal levels by 6 months. It was clear that a 6-month recovery period from subchronic exposure to 2,3,7,8-TCDD at a dose of 1.0 µg/kg/week was not sufficient for complete reversal of 2,3,7,8-TCDD-induced porphyria.

In addition to the above laboratory studies, Strik and de Wit (1980) attempted to investigate the toxicologic effect on rabbits of exposure to a natural environment that was contaminated with 2,3,7,8-TCDD. Groups of 20 female rabbits and 1 male rabbit were housed for 5 months in pens, located in five separate areas, on soil that had been contaminated with 2,3,7,8-TCDD. The soil had been cleaned by replacement or cultivation before initiation of the study. The levels of 2,3,7,8-TCDD before cleaning were from 0.8-23.2 µg/m^3; however, the levels of contamination after cleaning were not determined. At the end of 5 months liver histology, including the localization of porphyrin, was examined, and the levels of cytochrome P-450 and P-420 were determined along with urinary levels of total porphyrin, creatinine and D-glucaric-acid. All of the parameters examined were considered to be within the normal range. Since exposure data were not available, the negative results of this study cannot be compared with the controlled subchronic laboratory studies already described.

Information on the subchronic toxicity of HxCDD was provided in a preliminary range-finding study for a chronic bioassay conducted by NTP (1980d) on a 1-2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-HxCDD. Osborne-Mendel rats and $B6C3F_1$ mice in groups of 10 males and 10 females were administered the HxCDD mixture in corn oil-acetone (9:1) by gavage twice a week for 13 weeks. The total weekly doses given rats were 0.0, 2.5, 5, 10, 50 and 100 μg/kg, while mice received 0.0, 1.25, 2.5, 5, 10 and 50 μg/kg. At week 10 of the study, the body weight in rats was decreased in a dose-related manner to a maximum of ~20% in the high-dose group. In mice, body weight was also decreased 10-20% in the treated animals; however, there appeared to be no correlation with dose. At the end of the study the animals were killed and necropsies were performed on selected animals. In both species liver pathology was observed, with threshold to moderate hepatotoxicity occurring at doses of 5 and 10 μg/kg/week for male and female rats, respectively, and at 10 μg/kg/week for both sexes of mice. At higher exposures, splenic hyperplasia and cortical atrophy of the thymus were also detected in rats. In rats it was unclear whether the low-dose animals were free of any pathologic findings or none were subjected to necropsy. In mice it was stated that no changes were observed in males exposed to 2,3,7,8-TCDD at 1.25 μg/kg/week or in females exposed to 1.25 or 2.5 μg/kg/week. Although the data are limited, it appears that the same target organs are sensitive to the toxic effects of both 2,3,7,8-TCDD and this mixture of HxCDD.

In addition, a second subchronic range finding study conducted by NTP (1980c) evaluated the dermal toxicity of the above mixture of HxCDD. Groups of 10 male and 10 female Swiss-Webster mice were treated by dermal application 3 times/week for 13 weeks. The doses used were from 0.01-50 μg/

application with the test compound dissolved in acetone. There was 100% mortality in the 25 and 50 µg/application groups and 80% mortality in the 10 µg/application group. On histologic examination, there were signs of liver damage at the lowest dose tested in both sexes; however, the incidence and degree of damage were not well correlated to the dose applied.

Chronic Toxicity. In rats and mice the toxic effects of chronic exposure to 2,3,7,8-TCDD are summarized in Table V-5. These studies were predominately designed to assess the carcinogenicity of 2,3,7,8-TCDD and the observations of non-neoplastic systemic toxicity are, therefore, limited.

Van Miller et al. (1977a,b) fed groups of 10 male Sprague-Dawley rats diets containing 1, 5, 50, 500, 1000, 5000, 50,000, 500,000 or 1,000,000 ppt 2,3,7,8-TCDD (10^{-3} µg/kg diet) for 78 weeks in order to determine the potential toxic and carcinogenic effects of 2,3,7,8-TCDD. The authors estimated that these dietary levels corresponded to doses of 0.0003, 0.001, 0.01, 0.1, 0.4, 2.0, 24, 240 or 500 µg 2,3,7,8-TCDD/kg bw/week, respectively. All animals that received diets containing $\geq$1000 ppt ($\geq$0.4 µg/kg bw/week) were dead by the end of the study (95 weeks). It appeared that diets containing $\geq$1000 ppt (>0.4 µg/kg bw/week) 2,3,7,8-TCDD definitely increased mortality. The three highest dietary levels (50,000, 500,000 and 1,000,000 ppt) were acutely toxic, causing death between the second and fourth weeks of the study, with severe liver necrosis, thymic atrophy and cessation of growth. Conclusions could not be drawn concerning the effects of lower doses (lower than 500 ppt) on survival because of the small sample sizes and the high mortality in the control group (6 of 10). In the groups receiving <5000 ppt, weight gain was significantly depressed only in the

TABLE V-5

Effects of Chronic Exposure to 2,3,7,8-TCDD in Laboratory Rodents

Species/Strain	Sex/No.	Dose	Treatment Schedule	Duration of Study	Parameters Monitored	Effects of Treatment[a]	Reference
Rat/Sprague-Dawley	male/10	0.0 ppt	NA	95 weeks	survival	40% survived until 95 weeks, the first death occurred at week 68	Van Miller et al., 1977a,b
	male/10	1 ppt	continous in diet for 78 weeks	95 weeks	survival	80% survived until 95 weeks, the first death occurred at week 86	
	male/10	5 ppt	continuous in diet for 78 weeks	95 weeks	survival	60% survived until 95 weeks, the first death occurred at week 33	
	male/10	50 ppt	continuous in diet for 78 weeks	95 weeks	survival	60% survived until 95 weeks, the first death occurred at week 69	
	male/10	500 ppt	continuous in diet for 78 weeks	95 weeks	survival	50% survived until 95 weeks, the first death occurred at week 17	
	male/10	1000 and 5000 ppt	continuous in diet for 78 weeks	95 weeks	survival	No animals survived until 95 weeks, the first death occurred at week 31	
	male/10	50,000, 500,000 and 1,000,000 ppt	continuous in diet for 78 weeks	95 weeks	survival	No animals survived until 95 weeks, the first deaths occurred at weeks 2 and 3	

TABLE V-5 (cont.)

Species/Strain	Sex/No.	Dose	Treatment Schedule	Duration of Study	Parameters Monitored	Effects of Treatment*	Reference
Rat/Sprague-Dawley	M and F/ 50 and 50	~2193 ppt (0.1 μg/kg/day)	continuous in diet for 2 years	2 years	extensive histopathology, hematology, urine analyses, and clinical chemistry	Cumulative mortality, increased (F); Body weight gain, decreased (M,F); Red blood cell count, decreased (M,F); Packed cell volume, decreased (M,F); Hemoglobin, decreased (M,F); Reticulocytes, increased (M,F); White blood cell count, decreased (F); Serum glutamic pyruvic transaminase, increased (F); G-Glutamyl transferase, increased (F); Alkaline phosphatase, increased (F); Urinary coproporphyrin, increased (F); Urinary uroporphyrin, increased (F); Urinary delta-aminolevulinic acid, increased hepatic degeneration, increased (M,F)	Kociba et al., 1978a, 1979
	M and F/ 50 and 50	~208 ppt (0.01 μg/kg/day)	continuous in diet for 2 years	2 years	extensive histopathology, hematology, urine analyses and clinical chemistry	Urinary coproporphyrin, increased (F); Urinary uroprophyrin, increased (F); Hepatic degeneration, increased (M,F)	
	M and F/ 50 and 50	~22 ppt (0.001 μg/kg/day)	continuous in diet for 2 years	2 years,	extensive histopathology, urine analyses and clinical chemistry	No differences from values obtained from control animals	

TABLE V-5 (cont.)

Species/Strain	Sex/No.	Dose	Treatment Schedule	Duration of Study	Parameters Monitored	Effects of Treatment*	Reference
Rat/Osborne-Mendel	M and F/ 75 and 75	0.0 μg/kg/week	NA	106 weeks	extensive histopathology	Toxic hepatitis; 0/74 (M), 0/75 (F)	NTP, 1980a
	M and F/ 50 and 50	0.5 μg/kg/week	administered by gavage biweekly for 104 weeks	107 weeks	extensive histopathology	Toxic hepatitis; 14/50 (M), 32/50 (F)	
	M and F/ 50 and 50	0.05 μg/kg/week	administered by gavage biweekly for 104 weeks	107 weeks	extensive histopathology	Toxic hepatitis; 0/50 (M), 1/50 (F)	
	M and F/ 50 and 50	0.01 μg/kg/week	administered by gavage biweekly for 104 weeks	107 weeks	extensive histopathology	Toxic hepatitis; 1/50 (M), 0/50 (F)	
Mice/B6C3F1	M and F/ 75 and 75	0.0 μg/kg/week	NA	105-106 weeks	extensive histopathology	Toxic hepatitis; 1/73 (M), 0/73 (F)	NTP, 1980a
	M and F/ 50 and 50	0.5 μg/kg/week (M); 2.0 μg/kg/week (F)	administered by gavage biweekly for 104 weeks	107 weeks	extensive histopathology	Toxic hepatitis; 44/50 (M), 34/47 (F)	
	M and F/ 50 and 50	0.05 μg/kg/week (M); 0.2 μg/kg/week (F)	administered by gavage biweekly for 104 weeks	107 weeks	extensive histopathology	Toxic hepatitis; 3/49 (M), 2/48 (F)	
	M and F/ 50 and 50	0.01 μg/kg/week (M); 0.04 μg/kg/week (F)	administered by gavage biweekly for 104 weeks	107 weeks	extensive histopathology	Toxic hepatitis; 5/44 (M), 1/50 (F)	
Mice/Swiss	M/38	0.0 μg/kg/week	NA	588 days	histology on all organs	Dermatitis and amyloidosis; 0/38	Toth et al., 1978, 1979
	M/44	0.007 μg/kg/week	administered by gavage weekly for 1 year	649 days	histology on all organs	Dermatitis and amyloidosis; 5/44	
	M/44	0.7 μg/kg/week	administered by gavage weekly for 1 year	633 days	histology on all organs	Dermatitis and amyloidosis; 10/44	
	M/43	7.0 μg/kg/week	administered by gavage weekly for 1 year	424 days	histology on all organs	Early mortality, dermatitis and amyloidosis; 17/43	

*Results of statistical analysis were not provided.

NA = Not applicable

5000 ppt group. The only histopathologic changes reported in these groups were neoplastic changes (see Carcinogenicity).

Kociba et al. (1978a, 1979) maintained male and female Sprague-Dawley rats (50/sex/dose) on diets containing levels of 2,3,7,8-TCDD that resulted in doses of 0.001, 0.01 or 0.1 µg/kg/day. Increased mortality was observed in the high-dose females. In the groups receiving 0.01 or 0.1 µg/kg/day, treatment-related changes were observed in hematologic, clinical chemistry and urinary analysis values. Urinary excretion rates of coproporphyrin and uroporphyrin were increased in these groups. Histologic examination revealed degenerative, necrotic and inflammatory changes in the liver. The NOEL in this study was 0.001 µg/kg bw/day.

Toxic hepatitis (lipidosis and hydropic degeneration of the cytoplasm of the hepatocytes) was observed in both sexes of Osborne-Mendel rats receiving 2,3,7,8-TCDD by gavage in corn oil:acetone (9:1) (0.25 or 0.025 µg/kg bw), twice/week for 104 weeks (NTP, 1980a). No other non-neoplastic lesions were observed, even though extensive histological examinations were performed. This study demonstrated a NOAEL of 0.05 µg/kg bw/week for hepatitis.

In this same study, $B6C3F_1$ mice were treated biweekly for 104 weeks with 2,3,7,8-TCDD in corn oil:acetone (9:1). Males were given 0.0, 0.01, 0.05 or 0.5 µg/kg bw/week and females were given 0.0, 0.04, 0.2 or 2.0 µg/kg bw/week. Toxic hepatitis was observed in control and treated groups, but appeared to be significantly elevated only in the high dose groups (NTP, 1980a).

In another study, Toth et al. (1978, 1979) intubated male Swiss mice with 0.0, 0.007, 0.7 or 7.0 µg 2,3,7,8-TCDD/kg bw/week for 1 year. Amyloidosis of the kidney, spleen and liver, and dermatitis were observed in all three treatment groups. This suggests a LOAEL of 0.007 µg/kg bw/week in mice in this study, but a NOAEL was not established.

Some information on the toxicity of chronic dermal exposure can be obtained from the NTP (1980b) dermal carcinogenicity study in Swiss-Webster mice. Thirty males were treated with 0.01 µg 2,3,7,8-TCDD/application and 30 females were treated with 0.005 µg/application, 3 times/week for 104 weeks. Vehicle-treated and untreated controls were included in the experiment. Although no effects on body weight were observed, treated male mice had a significant shortening of lifespan, and treated females showed non-neoplastic hepatic lesions (hepatic cytomegaly and inflammatory areas).

Target Organ Toxicity

Hepatic Effects. As previously described, when administered lethal doses of 2,3,7,8-TCDD, guinea pigs and monkeys have few or no histopathologic changes in the liver, whereas rats and mice have extensive histopathologic effects in this organ. Gupta et al. (1973) and Grieg et al. (1973) observed marked distortion of liver architecture in rats given 50 or 100 µg 2,3,7,8-TCDD/kg, with marked necrosis of hepatocytes. The livers of mice given lethal doses of 2,3,7,8-TCDD also had necrosis (Vos et al., 1974). According to Jones and Grieg (1975), centrilobular necrosis, bile duct proliferation and lipid accumulation were more severe in mice than in rats. Livers of mice contained excess amounts of porphyrin (McConnell et al., 1978a).

Light microscopic, ultrastructural and histochemical changes in the livers of rats given 2,3,7,8-TCDD orally have been described by Fowler et al. (1973), Jones and Butler (1974) and Jones (1975). Fowler et al. (1973) administered 0.0, 5.0 or 25.0 µg 2,3,7,8-TCDD/kg bw by gavage to groups of 30 male rats. The rats were sacrificed 1, 3, 6, 9, 16 or 28 days after treatment. Major ultrastructural changes occurred in the cells near the bile canaliculi in both treatment groups. A dose-related increase in the smooth and rough endoplasmic reticulum was observed starting on day 3, reaching a maximum on days 6-9, and returned to normal by day 28.

Jones and Butler (1974) administered 200 µg 2,3,7,8-TCDD/kg bw to groups of 40 male and 40 female Porton rats and sacrificed 4 rats of each sex/week for 10 weeks. The major lesions observed were necrosis and proliferative changes in the liver. Degenerating cells were observed near the central vein, progressing to areas of focal necrosis by week 6 and central vein fibrosis with scattered necrosis by week 10. Additionally, hyperplasia of the viable cells, with many multinucleated cells, was prevalent by week 9. Jones (1975) gave 23 male Porton rats a single gavage dose of 200 µg 2,3,7,8-TCDD/kg in arachis oil, and 7 control rats received arachis oil alone. Loss of ATPase activity along the canalicular borders and increased activity in the sinusoids in the livers of rats were observed 3 days following treatment. The loss of ATPase activity persisted for at least 34-42 days, but returned to normal by 9 months.

Peterson et al. (1979a) investigated the effects of lower doses (10 or 25 µg/kg bw, oral) of 2,3,7,8-TCDD on ATPase activity in hepatocyte plasma membranes. Liver surface membranes were isolated from male Holtzman rats using a modified discontinuous sucrose gradient method on days 2, 10, 20 or 40 post-treatment. Both doses resulted in similar depression of Na/K-ATPase on days 2-40; however, Mg^{++}-ATPase was depressed to this extent only in the high-dose groups. In the low-dose group, Mg^{++}-ATPase activity was decreased on day 20, but returned to normal levels by day 40 post-treatment. 2,3,7,8-TCDD did not inhibit ATPase activity in vitro, nor did the decrease in ATPase activity correlate with 2,3,7,8-TCDD induced food deprivation. There was, however, a correlation between liver surface membrane activity, bile flow and biliary excretion of ouabain in vivo. In similar experiments, Hwang (1973) reported that bile flow was stimulated, but indocyanine green excretion was inhibited, in male CD rats following treatment with 5 or 25 μg 2,3,7,8-TCDD/kg bw by gavage.

Peterson et al. (1979b) investigated the relationship between ATPase activity and biliary excretion of ouabain in perfused liver from rats. They discovered that liver surface membrane ATPase activity and ouabain excretion could be segregated by cotreatment with the use of spironolactone, chemicals that increase bile flow, or pregnenolone-16-α-carbonitrile, indicating that this ATPase activity was not directly involved in ouabain transport. The ability of 2,3,7,8-TCDD to inhibit biliary excretion has been demonstrated to correlate with the species sensitivity to the hepatotoxic effects of 2,3,7,8-TCDD (Seefeld et al., 1979, 1980). Yang et al. (1977) reported that the effect of 2,3,7,8-TCDD on biliary transport is compound dependent. In these studies, ouabain excretion, a neutral compound, and bile flow were inhibited, while the excretion of the organic anions phenol-3,6-dibromophthalein and sulfobromophthalein were essentially unchanged.

Cunningham and Williams (1972) detected a decrease in *in vivo* lipid formation in the liver of male Wistar rats receiving 10 μg 2,3,7,8-TCDD/kg bw, based on [^{3}H]sodium acetate incorporation. They determined that the synthesis of triglycerides, diglycerides and phospholipids were all depressed. Despite this inhibition of lipid synthesis, Albro et al. (1978) found an increase in total hepatic lipids in rats 13 days following treatment with 50 μg 2,3,7,8-TCDD/kg bw. Levels of free fatty acids and cholesterol esters were increased while levels of phospholipids, free cholesterol and triglycerides remained constant. When a sublethal dose, 10 μg/kg bw, was administered, triglycerides and fatty acids were increased and cholesterol esters were decreased. These changes were explained by mobilization of body fat, decreased lysosomal acid lipase, and increased lipid peroxidation.

Goldstein et al. (1978) determined that 4 weekly doses of 25 μg 2,3,7,8-TCDD/kg bw or higher would result in increased hepatic porphyrin levels in male C57Bl mice. Smith et al. (1981) found that strain differences in sensitivity between C57Bl and DBA/2 mice given a single gavage dose of 75 μg 2,3,7,8-TCDD/kg correlated with the ability of 2,3,7,8-TCDD to induce increases in hepatic porphyrins; however, there was no correlation between toxicity and increased porphyrins between sexes within strains. Rats are less sensitive to 2,3,7,8-TCDD induced porphyria, with increases in porphyrin levels occurring only after subchronic exposure (≥0.01 mg/kg/week by gavage for 6 months) (Cantoni et al., 1981). The major factors involved in 2,3,7,8-TCDD-induced porphyria have been identified as an increase in α-aminolevulinic acid synthetase (Goldstein et al., 1978) and a decrease in uroporphyrinogen decarboxylase activity (Smith et al., 1981; Sweeney and Jones, 1978).

A number of groups investigated the effect of 2,3,7,8-TCDD on hepatic DNA synthesis. Grieg et al. (1974) measured the incorporation of [³H]thymidine into the hepatic DNA of male and female Porten rats following partial hepatectomy. No effect of 2,3,7,8-TCDD pretreatment (10 or 200 mg/kg bw) was noted. In *in vitro* studies, however, Conway and Matsumura (1975) and Dickens et al. (1981) demonstrated increased [³H]thymidine incorporation into DNA in liver slices obtained from rats which had been pretreated with 5 µg 2,3,7,8-TCDD/kg bw. 2,3,7,8-TCDD is also a potent inducer of hepatic microsomal enzymes (see Enzyme Induction in Chapter VII).

Immunological Effects. The consistent observation of thymic atrophy in early acute toxicity studies suggested that 2,3,7,8-TCDD may alter the immune response and prompted investigations of immunotoxicity, summarized in Table V-4. The results suggest that 2,3,7,8-TCDD interferes with the immunologic capability of the animals tested. Vos et al. (1973) investigated the effect of orally administered 2,3,7,8-TCDD on the cell-mediated and humoral immune response of $B6D2F_1$ mice, CD rats and Hartley guinea pigs. Pretreatment with 0.04 µg 2,3,7,8-TCDD/kg bw/week reduced the development of skin hypersensitivity to *Mycobacterium tuberculosis* in guinea pigs; no effect on this parameter was seen in rats. Tetanus antitoxin levels were reduced in guinea pigs receiving 0.2 µg 2,3,7,8-TCDD/kg bw/week. 2,3,7,8-TCDD has also been demonstrated to inhibit cell-mediated immunity in "graft-versus-host" experiments utilizing spleen cells from mice which had received 4 weekly oral doses of 0, 0.2, 1.0 or 5.0 µg 2,3,7,8-TCDD/kg bw (Vos et al., 1973).

Vos and Moore (1974) and Moore and Vos (1974) administered 1 or 5 µg 2,3,7,8-TCDD/kg bw to female F-344 rats on days 11 and 18 of gestation and days 0, 7 and 14 postpartum. This treatment resulted in prolonged times until graft rejection, decreased spleen cell graft-versus-host activity and decreased binding response to phytohemogglutinin, but an increased humoral immune response to conconavalin A in the pups.

Thigpen et al. (1975) determined the effect of 2,3,7,8-TCDD on host resistance to injection by Salmonella bern in male SPF C57Bl/6Jfh mice, an effect largely mediated by thymus-derived lymphocytes. Pretreatment with ≥1 µg/kg bw/week by gavage resulted in an increase in mortality and a decrease in time until infection. The NOAEL was determined to be 0.5 µg/kg bw. Pretreatment with 2,3,7,8-TCDD has also been demonstrated to enhance the susceptibility of mice to E. coli endotoxin (Vos et al., 1978a).

Thomas and Hinsdill (1979) maintained female Swiss-Webster mice on diets containing 1, 2.5, 5, 10 or 20 ppb 2,3,7,8-TCDD (1, 2.5, 5, 10 or 20 µg/kg diet) from 4 weeks before mating until 3 weeks postpartum. Thymus weight was decreased in the pups from groups receiving ≥2.5 ppb, along with a decreased number of plaque-forming cells reactive to sheep red blood cells and an increased susceptibility to S. typhimurium. This treatment did not, however, lower the humoral content of anti-RBC antibodies, affect the response to E. coli LPS or L. monocytogenes, affect nitrogen-induced lymphocyte proliferation, or alter the response of B- and T-cells to conconcanavalin A in vitro. Similar results have been reported in Fisher/Wistar rats (Faith and Luster, 1979; Luster et al., 1980).

Hinsdill et al. (1980) found that feeding Swiss-Webster mice diets containing 100 ppb (100 μg/kg diet) 2,3,7,8-TCDD for 5 weeks increased serum β-globulins, but suppressed total serum protein, γ-globulin and albumin. A decreased immune response to tetanus toxoid, sheep red blood cells, _S. typhimurium_ and _L. monocytogenes_ and a lowered contact sensitivity to DNFB were noted at dietary concentrations as low as 10 ppb (10 μg/kg diet). Young animals were slightly more susceptible to the immunosuppressive effects of 2,3,7,8-TCDD than were older animals. Vos and Moore (1974) also reported that 1-month-old mice are more sensitive to 2,3,7,8-TCDD than are 4-month-old mice.

Mantovani et al. (1980) administered single i.p. doses of 1, 2, 6 or 30 μg 2,3,7,8-TCDD/kg bw to C57Bl/6J mice. This treatment resulted in a decreased total number of macrophages and splenic natural killer cells, but did not affect the cytostatic or cytocidal activity of the remaining cells. McConnell et al. (1978a) suggested that the decrease in peripheral cell counts may be the result of the hypocellularity seen in the bone marrow of 2,3,7,8-TCDD-treated mice.

Other Organ Systems. One of the characteristic effects of 2,3,7,8-TCDD is the loss of body weight. Because decreased food consumption does not totally account for this finding, the potential effects of 2,3,7,8-TCDD on intestinal absorption were of interest. Madge (1977) investigated the effect of 2,3,7,8-TCDD on intestinal absorption in CD-1 mice using the everted intestinal sac technique. Treatment of the mice with single gavage doses of 0, 10, 25, 75, 150, 200 or 300 μg 2,3,7,8-TCDD/kg bw decreased the in vitro intestinal absorption of D-glucose, but did not affect absorption of D-galactose, L-argenine or L-histidine. These measurements were made 7 days after treatment. In a similar study, Ball and Chhabra (1981) reported that 2,3,7,8-TCDD reduced the absorption of D-glucose and leucine in Sprague-Dawley rats.

Manis and Kim (1979a,b) found that 22-84 µg 2,3,7,8-TCDD stimulated intestinal transport of iron in male Sprague-Dawley rats and in an unidentified strain of mice. Gavage administration was more effective than i.p. injection. This stimulation occurred in the duodenum, but the distal segment of the intestine was unaffected. In parallel experiments, calcium transport was decreased and galactose and proline were unaffected by prior exposure to 2,3,7,8-TCDD.

Pegg et al. (1976) and Hook et al. (1977) investigated the effect of 10 or 25 µg 2,3,7,8-TCDD on the in vitro function of renal cortical slices obtained from male Sprague-Dawley rats 3 or 7 days following intubation. Anion transport, as measured by accumulation of p-aminohippuric acid, was decreased by N-methyl-nicotinamide accumulation, at both dose levels. In the in vivo studies, sodium reabsorption was within the normal range and ammoniogenesis and gluconeogenesis were unaffected, even when the rats were made acidotic. The authors concluded that the observed decrease in kidney function was a result of the generally poor condition of the treated animals and not a direct effect of 2,3,7,8-TCDD.

Grieg et al. (1974) found that intraperitoneal administration of 10 µg 2,3,7,8-TCDD/kg bw inhibited the DNA synthesis stimulated by folate (250 mg folic acid/kg) or lead acetate (40 mg Pb^{+2}/kg) in the kidneys of male and female Porton strain rats. This inhibition did not involve direct interaction with DNA or the inhibition of protein synthesis, but may involve the aromatic oxidases of liver microsome.

Zinkl et al. (1973) observed a number of hematologic clinical chemistry changes in female CD rats given 30 daily doses of 0.1, 1.0 or 10 µg 2,3,7,8-TCDD/kg bw; however, they concluded that these changes simply reflected damage to other organs, such as the liver, and hemoconcentration. The only other major effect observed was thrombocytopenia, which was also observed in mice and female guinea pigs. Lymphopenia was observed in guinea pigs that received a single dose of 1, 10 or 50 µg/kg bw.

Poli et al. (1980) investigated the induction of hyperlipidemia in male Sprague-Dawley rats following a single i.p. injection of 2.5, 5, 10 or 20 µg 2,3,7,8-TCDD/kg bw. There was a dose-related increase in total plasma cholesterol and high density lipoprotein cholesterol by 21 days post-treatment, but triglycerides and low and very low density lipoproteins were unaffected. In contrast, hyperlipidemia in male Hartley guinea pigs given a single i.p. injection of 2 µg/kg bw was characterized by increases in low and very low density lipoproteins.

Other Effects

Carcinogenicity. 2,3,7,8-TCDD has been tested for carcinogenicity in rats and mice by administering the compound in the diet and by gavage.

Also, the tumor incidence in native mice inhabiting an area with heavy exposure to the herbicide Agent Orange has been assessed and compared with mice from an uncontaminated habitat. The results of these bioassays are summarized in Table V-6. Along with studies using the oral route, 2,3,7,8-TCDD has been tested for tumorigenicity by dermal application (Table V-7). Using the skin two-stage tumorigenicity model, 2,3,7,8-TCDD has been tested for promoting and initiating activity as well as anticarcinogenic activity. Other model systems have been used to a more limited extent in studies of the effect of 2,3,7,8-TCDD on the carcinogenic potential of chemical carcinogens.

In a limited study, Van Miller (1977a,b) maintained small groups of male Sprague-Dawley rats on diets containing 2,3,7,8-TCDD. The animals, in groups of 10, were fed diets containing 0.0, 0.001, 0.005, 0.05, 0.5, 1.0, 5.0, 50, 500 or 1000 ppb (μg/kg diet) of 2,3,7,8-TCDD for 78 weeks. As determined from the food consumption of two animals from each group, these exposure levels corresponded to doses of 0.0, 0.0003, 0.001, 0.01, 0.1, 0.4, 2.0, 24, 240 and 500 μg/kg/week, respectively. Animals exposed to $\geq$50 ppb died before tumor development could occur. At week 65 of treatment, all surviving animals were examined by laparotomy, and biopsy samples were obtained from any gross tumors. Following termination of treatment, the animals were observed for an additional 17 weeks before and until sacrificing all surviving animals. At necropsy, performed on animals killed when moribund, found dead, or killed at termination of the study, the animals were examined for both gross and microscopic lesions.

TABLE V-6

Carcinogenicity Bioassays of 2,3,7,8-TCDD Administration by the Oral Route

Exposure Route	Species/Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Vehicle	Tumor Type	Tumor Incidence	p Value	Reference
Gavage	rats/ Osborne-Mendel	M	0.0 μg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	follicular-cell adenomas of the thyroid, carcinoma of the thyroid	1/69 0/69	=0.006	NTP, 1980a
			0.01 μg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	follicular-cell adenomas of the thyroid, carcinoma of the thyroid	5/48 0/48	=0.042	
			0.05 μg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	follicular-cell adenomas of the thyroid, carcinoma of the thyroid	6/50 2/50	=0.021	
			0.5 μg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	follicular-cell adenomas of the thyroid, carcinoma of the thyroid	10/50 1/50	=0.001	
Gavage	rats/ Osborne-Mendel	F	0.0 μg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	neoplastic nodule of the liver, hepatocellular carcinoma of the liver	5/75 0/75	<0.001	NTP, 1980a
			0.01 μg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	neoplastic nodule of the liver, hepatocellular carcinoma of the liver	1/49 0/49	NS	
			0.05 μg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	neoplastic nodule of the liver, hepatocellular carcinoma of the liver	3/50 0/50	NS	
			0.5 μg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	neoplastic nodule of the liver, hepatocellular carcinoma of the liver	12/49 3/49	=0.006	
Gavage	mice/B6C3F$_1$	M	0.0 μg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma	8/73	=0.002	NTP, 1980a
			0.01 μg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma	9/49	NS	
			0.05 μg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma	8/49	NS	
			0.5 μg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma	17/50	=0.002	

TABLE V-6 (cont.)

Exposure Route	Species/Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Vehicle	Tumor Type	Tumor Incidence	p Value	Reference
Gavage	mice/B6C3F$_1$	F	0.0 μg/kg/week	104 weeks	105 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma, follicular-cell adenomas of the thyroid	1/73 0/69	=0.008 =0.016	NTP, 1980a
			0.04 μg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma, follicular-cell adenomas of the thyroid	2/50 3/50	NS NS	
			0.2 μg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma, follicular-cell adenomas of the thyroid	2/48 1/47	NS NS	
			2.0 μg/kg/week	104 weeks	107 weeks	corn oil-acetone (9:1)	hepatocellular carcinoma, follicular-cell adenomas of the thyroid	6/47 5/46	=0.014 =0.009	
Oral	rat/ Sprague-Dawley	M	0.0 ppb	78 weeks	95 weeks	in diet	all tumors^a	0/10	NR	Van Miller et al., 1977a,b
			0.001 ppb	78 weeks	95 weeks	in diet	all tumors^a	0/10	NR	
			0.005 ppb	78 weeks	95 weeks	in diet	all tumors^a	5/10	NR	
			0.05 ppb	78 weeks	95 weeks	in diet	all tumors^a	3/10	NR	
			0.5 ppb	78 weeks	95 weeks	in diet	all tumors^a	4/10	NR	
			1.0 ppb	78 weeks	95 weeks	in diet	all tumors^a	4/10	NR	
			5.0 ppb	78 weeks	95 weeks	in diet	all tumors^a	7/10	NR	
Oral	rat/ Sprague-Dawley	M	0.0 μg/kg/day	105 weeks	105 weeks	in diet	squamous cell carcinoma of the hard palate, squamous cell carcinoma of the tongue, adenoma of the adrenal cortex	0/85 0/85 0/85	NS NS NS	Kociba et al., 1978a,b
			0.001 μg/kg/day	105 weeks	105 weeks	in diet	squamous cell carcinoma of the hard palate, squamous cell carcinoma of the tongue, adenoma of the adrenal cortex	0/50 1/50 0/50	NS NS NS	

TABLE V-6 (cont.)

Exposure Route	Species/Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Vehicle	Tumor Type	Tumor Incidence	p Value	Reference
Oral	rat/ Sprague-Dawley	M	0.01 μg/kg/day	105 weeks	105 weeks	in diet	squamous cell carcinoma of the hard palate,	0/50	NS	Kociba et al., 1978a,b
							squamous cell carcinoma of the tongue,	1/50	NS	
							adenoma of the adrenal cortex	2/50	NS	
			0.1 μg/kg/day	105 weeks	105 weeks	in diet	squamous cell carcinoma of the hard palate,	4/50	<0.05	
							squamous cell carcinoma of the tongue,	3/50	<0.05	
							adenoma of the adrenal cortex	5/50	<0.05	
Oral	rat/ Sprague-Dawley	F	0.0 μg/kg/day	105 weeks	105 weeks	in diet	hepatocellular carcinoma,	0/86	NS	Kociba et al., 1978a,b
							squamous cell carcinoma of the tongue,	0/86	NS	
							squamous cell carcinoma of the lung	0/86	NS	
			0.001 μg/kg/day	105 weeks	105 weeks	in diet	hepatocellular carcinoma,	0/50	NS	
							squamous cell carcinoma of the tongue,	0/50	NS	
							squamous cell carcinoma of the lung	0/50	NS	
			0.01 μg/kg/day	105 weeks	105 weeks	in diet	hepatocellular carcinoma,	2/50	NS	
							squamous cell carcinoma of the tongue,	1/50	NS	
							squamous cell carcinoma of the lung	0/50	NS	
Oral	rat/ Sprague-Dawley	F	0.1 μg/kg/day	105 weeks	105 weeks	in diet	hepatocellular carcinoma,	11/49	<0.05	Kociba et al., 1978a,b
							squamous cell carcinoma of the tongue,	4/49	<0.05	
							squamous cell carcinoma of the lung	7/49	<0.05	

TABLE V-6 (cont.)

Exposure Route	Species/Strain	Sex	Dose or Exposure	Duration of Treatment	Duration of Study	Vehicle	Tumor Type	Tumor Incidence	p Value	Reference
Gavage	mice/ Swiss/H/Riop	M	0.0 μg/kg/week	365 days	580 days	sunflower oil	liver tumors[b]	7/38	NS	Toth et al., 1979
			0.007 μg/kg/week	365 days	649 days	sunflower oil	liver tumors[b]	13/44	NS	
			0.7 μg/kg/week	365 days	633 days	sunflower oil	liver tumors[b]	21/44	<0.01	
			7.0 μg/kg/week	365 days	424 days	sunflower oil	liver tumors[b]	13/43	NS	
Oral	mice/ Peromyscus polionotus	M,F	0.0012 μg/kg/day	NA	NA	contaminated soil	liver	0/15	NS	Cockerham et al., 1980
			0.0 μg/kg/day	NA	NA	contaminated soil	liver	0/15	NS	

[a] No single target organ for cancer was outstanding.

[b] Includes hepatomas and hepatocellular carcinomas.

NR = Not reported

NS = Not significant

TABLE V-7

Carcinogenicity Bioassays of 2,3,7,8-TCDD Administered by the Dermal Route

Species	Sex	Dose[b]	Duration of Exposure	Target Organ	Tumor Type	Tumor Incidence
Mice	M	0.01 μg/application	104 weeks	integumentary system	fibrosarcoma	6/28
		0.0 μg/application (vehicle control)	104 weeks	integumentary system	fibrosarcoma	3/42
		0.0 μg/application (untreated control)	NA	integumentary system	fibrosarcoma	0/28
	F	0.005 μg/application	104 weeks	integumentary system	fibrosarcoma	8/28
		0.0 μg/application (vehicle control)	104 weeks	integumentary system	fibrosarcoma	2/41
		0.0 μg/application (untreated control)	NA	integumentary system	fibrosarcoma	1/27

[a] Source: NTP, 1980b

[b] The compound was applied 3 times/week in 100 μℓ of acetone.

NA = Not applicable

All animals in groups maintained on diets containing 1-1000 ppb of 2,3,7,8-TCDD were dead by week 90 of treatment with the first deaths in groups at the 1000 and 1 ppb levels observed at 2 weeks and 31 weeks of treatment, respectively (Van Miller et al., 1977a,b). Animals exposed to 0.001-0.5 ppb of 2,3,7,8-TCDD had similar food consumption (20 g/day) and survival (4/10, 8/10, 6/10, 6/10, 5/10 survived in the 0, 0.001, 0.005, 0.05 and 0.5 ppb treatment groups, respectively) as control animals. All treated and control animals had extensive senile degenerative changes in the kidneys. Complete necropsies were done and samples of tissues were taken for microscopic examination from the control groups and each treatment group.

Special staining methods were used as an aid in the diagnosis of neoplasms. Various benign and malignant tumors were found in each treatment group. No tumors were observed in the controls (Table V-8).

Statistically significant increases of squamous cell tumors of the lungs and neoplastic nodules of the liver were observed in rats ingesting 5 ppb TCDD (Tables V-9 and V-10). In addition, two animals in the 5 ppb dose group and one animal in the 1 ppb dose group had liver cholangiocarcinomas, which are rare in Sprague-Dawley rats. These results provide evidence of a carcinogenic effect.

The observation of no tumors of any kind in the controls is unusual for Sprague-Dawley rats. In addition, the reporting of the study was not extensive. These factors may tend to lessen the reliance which can be placed on the positive results of this study. However, this study is suggestive of a carcinogenic response upon exposure to TCDD in rats.

TABLE V-8

2,3,7,8-TCDD Intake and Mortality in Male Sprague-Dawley Rats[a]

Dose[b] (ppb)	Weekly Dose/Rat (μg/kg bw)	Week of First Death	Number of Rats Dead at 95th Week
0.0	--	68	6/10 (60%)
0.001	0.0003	86	2/10 (20%)
0.005	0.001	33	4/10 (40%)
0.05	0.01	69	4/10 (40%)
0.5	0.1	17	5/10 (50%)
1	0.4	31	10/10 (100%)
5	2.0	31	10/10 (100%)

[a] Source: Adapted from Van Miller et al., 1977a,b

[b] Rats at 50, 500 and 1000 ppb dose levels were all dead within 4 weeks.

TABLE V-9

Benign and Malignant Tumors in Rats Ingesting 2,3,7,8-TCDD[a]

Dose[b]	Benign	Malignant	Number of Tumors	Number of Rats With Tumors
0	0	0	0	0/10 (0%)[c]
1 ppt	0	0	0	0/10 (0%)
5 ppt	1	5	6[d]	5/10 (50%)[e]
50 ppt	2	1	3[f]	3/10 (30%)
500 ppt	2	2	4[g]	4/10 (40%)[h]
1 ppb	0	4	5[i]	4/10 (40%)
5 ppb	8	2	10[j]	7/10 (70%)

[a] Source: Adapted from Van Miller et al., 1977a,b

[b] Rats at dose levels of 50, 500 and 1000 ppb were all dead within 4 weeks.

[c] 40 male rats used as controls for another study, received at the same time and kept under identical conditions, did not have neoplasms when killed at 18 months.

[d] 1 rat had ear duct carcinoma and lymphocytic leukemia
1 adenocarcinoma (kidney)
1 malignant histiocytoma (retroperitoneal)
1 angiosarcoma (skin)
1 Leydig cell adenoma (testis)

[e] 3 rats died with aplastic anemia

[f] 1 fibrosarcoma (muscle)
1 squamous cell tumor (skin)
1 astrocytoma (brain)

[g] 1 fibroma (striated muscle)
1 carcinoma (skin)
1 adenocarcinoma (kidney)
1 sclerosing seminoma (testis)

[h] 1 rat had a severe liver infarction

[i] 1 rat cholangiocarcinoma and malignant histiocytomas (retroperitoneal)
1 angiosarcoma (skin)
1 glioblastoma (brain)
1 malignant histiocytoma (retroperitoneal)

[j] 1 rat had squamous cell tumor (lung) and neoplastic nodule (liver)
2 cholangiocarcinomas and neoplastic nodules (liver)
3 squamous cell tumors (lung)
1 neoplastic nodule

TABLE V-10

Liver Tumors in Rats Ingesting 2,3,7,8-TCDD[a]

Dose (ppb)	Neoplastic Nodules	Cholangiocarcinomas	Squamous Cell Tumors of the Lungs
0	0/10 (0%)	0/10 (0%)	0/10
1	0/10 (0%)	1/10 (10%)	0/10
5	4/10 (40%) p=0.043	2/10 (20%)[b]	4/10 (40%) p=0.043

[a] Source: Adapted from Van Miller et al., 1977a,b

[b] The two animals had both neoplastic nodules of the liver and cholangiocarcinomas.

The second study employing oral administration to rats, performed by Kociba et al. (1978a,b), was more extensive. Groups of 100 Sprague-Dawley rats (50 males and 50 females) were exposed for 2 years to diets containing ~22, 208 and 2193 ppt of 99% pure 2,3,7,8-TCDD. The dietary levels were adjusted according to body weight in order to maintain 2,3,7,8-TCDD dose levels of 0.001, 0.01 or 0.1 μg/kg/day. The control group consisted of 172 rats (86 males and 86 females) maintained on unadulterated diets. During the study, the animals were palpated periodically for gross tumors and clinical chemistry analyses were performed on the blood and urine. Gross and histopathologic examinations of major organs were conducted in animals removed from the study before week 105, while extensive histopathologic examinations of a large number of organs and tissues were conducted on animals killed at the termination of the study.

Gross signs of 2,3,7,8-TCDD toxicity included significantly increased cumulative mortality in the high dose female rats, and lower body weights in the high dose male and female and medium dose female rats (p values not given). Hematologic values including packed cell volume and hemoglobin concentration were decreased ($p<0.05$) in both sexes at the 0.1 μg/kg/day dose, and urinary coproporphyrin and uroporphryin were increased ($p<0.05$) in females receiving 0.01 and 0.1 μg/kg/day. Statistically significant increases in tumors at several sites occurred in both male and female rats in the high dose group and liver lesions, including hepatocellular neoplastic nodules and lung lesions including focal alveolar hyperplasia in mid-dosed females. Tumors of the hard palate, tongue and adrenal cortex were increased in high-dosed males; tumors of the liver, tongue and lung were increased in high-dosed females. The most sensitive target organ appeared to be the liver of female rats with hepatocellular carcinoma incidences of 0/86, 0/50, 2/50 and 11/49 for the control, low, medium and high dose groups, respectively. There was no increase in the hepatocellular carcinoma incidence in male animals. Other tumors commonly observed in male and female rats were significantly ($p<0.05$) decreased in the treated animals (Table V-11). There was no increase in the neoplastic lesions in low-dosed males and females.

In a study to determine the carcinogenic potential of the herbicide 2,4,5-trichlorophenoxyethanol (2,4,5-TCPE), Toth et al. (1978, 1979) tested both this compound containing the known contaminant 2,3,7,8-TCDD and 2,3,7,8-TCDD alone by gavage in Swiss-H/Riop mice. Groups of 45 male mice were administered 2,3,7,8-TCDD dissolved in sunflower oil at dose levels of 0.007, 0.7 or 7.0 μg/kg bw once a week for 1 year. Other groups consisting of 100 male and 100 female mice were administered 2,4,5-TCPE contaminated with measured amounts of 2,3,7,8-TCDD suspended in 0.5% carboxymethylcellulose once a week for 1 year. Control groups administered each vehicle alone were included in the study for comparison. Following the treatment period, the animals were observed until moribund or until spontaneous death. At autopsy, animals were examined for tumors, and organs were obtained for histopathologic examination.

The only tumors that were observed to occur at a significantly greater incidence in either the 2,3,7,8-TCDD or the 2,4,5-TCPE/2,3,7,8-TCDD treatment groups compared with the appropriate vehicle control were tumors of the liver. These tumors consisted of both benign hepatomas and hepatocellular carcinomas. In the 2,3,7,8-TCDD treated mice, the incidence of liver tumors

TABLE V-11

Tumors That Were Significantly Decreased in Rats Following Exposure to 2,3,7,8-TCDD[a]

Sex	Tumor Type	Tumor Incidence			
		0.0 µg/kg/day	0.1 µg/kg/day	0.01 µg/kg/day	0.001 µg/kg/day
M	Acinar adenoma of the pancreas	14/85	2/50[b]	5/50	7/50
M	Pheochromocytoma of the adrenal	28/85	4/50[b]	10/50	6/50
M	Subcutaneous fibro-adenoma/fibroma/lipoma	10/85	6/50	5/50	1/50[b]
F	Benign tumors of the uterus	28/86	7/49[b]	11/50	12/50
F	Benign tumors of the mammary gland	73/86	24/49[b]	36/50	35/50
F	Carcinoma of the mammary gland	8/86	0/49[b]	4/50	4/50
F	Pituitary adenoma	43/86	12/49[b]	13/50	18/50

[a] Source: Kociba et al., 1978a

[b] The tumor incidence was significantly ($p<0.05$) different from the incidence in this control group.

was 7/38, 13/44, 21/44 and 13/43 for groups administered 0.0, 0.007, 0.7 and 7.0 μg/kg bw/week. The incidences of benign and malignant tumors were not enumerated separately. Only the 0.7 μg/kg/week group had a statistically significant (p<1%) increased incidence of tumors as compared with the control animals. The high dose group had a shorter lifespan, 424 days as compared with 588 days in the control group, and this may have affected tumor yield. The tumor incidence for mice receiving 2,4,5-TCPE containing 2,3,7,8-TCDD is presented in Table V-12. The authors concluded that both 2,4,5-TCPE and 2,3,7,8-TCDD were carcinogenic since an increased incidence of liver tumors was observed in groups receiving 2,4,5-TCPE even though the 2,3,7,8-TCDD level in one group was 0.007 μg/kg/day, which was shown to be nontumorigenic when 2,3,7,8-TCDD was administered alone. An exact quantitative comparison between the groups receiving 2,3,7,8-TCDD and the groups receiving the mixture is difficult to assess as a result of the use of two vehicles (sunflower oil and carboxymethylcellulose) with drastically different physical properties.

Under the National Toxicology Program, 2,3,7,8-TCDD has been tested for carcinogenicity by oral administration in Osborne-Mendel rats and $B6C3F_1$ mice (NTP, 1980a) and by dermal application to Swiss-Webster mice (NTP, 1980b). For both oral and dermal studies, the compound was custom synthesized and shown by analysis using gas chromatography to be 99.0-99.4% pure. The two major impurities tentatively identified by gas chromatography were trichlorodibenzo-p-dioxin and PeCDD, with HxCDD detected at levels of 0.1-0.2% by gas chromatography mass spectrometry. The chemical was protected from light and kept at room temperature until used to make stock solution at 3-month intervals. In both studies, the approximate maximal tolerated dose was determined by a preliminary subchronic toxicity study.

TABLE V-12

Tumor Incidence in Mice Treated with 2,4,5-TCPE Contaminated with 2,3,7,8-TCDD[a]

Group	TCPE[b] (mg/kg)	Treatment		Sex	Effective Number of Mice	Number of Tumor Bearing Mice	Number of Animals with Tumors of:				
		TCDD (μg/kg)	Vehicle[c] (mg/kg)				Liver (%)	Lung	Lymphomas	Other Organs	Average Lifespan
1	67.0	0.112 (1.6 ppm)	50	M	88	69	42[d] (18)	50	7	16	595
				F	83	61	7 (8)	52	15	25	652
2	70.0	0.007 (0.1 ppm)	50	M	98	78	57[e] (58)	18	11	16	571
				F	96	59	9 (9)	39	15	23	582
3		control		M	93	63	24 (26)	44	8	17	577
				F	84	57	4 (5)	41	23	13	639
4	7.0	0.07 (10 ppm)	50	M	93	79	25 (27)	38	18	22	641
				F	96	60	10 (10)	38	19	19	589
5	7.0	0.0007 (0.1 ppm)	50	M	94	77	23 (24)	50	23	17	660
				F	93	71	8 (9)	42	36	21	590
6	0.7	0.00007 (0.1 ppm)	50	M	97	78	24 (25)	51	20	17	643
				F	94	64	5 (5)	38	22	21	566
7	--	--	50	M	96	74	32 (33)	44	14	22	615
				F	84	55	4 (5)	38	18	17	565
8		control		M	96	78	32 (33)	38	22	15	651
				F	91	57	4 (4)	31	24	19	549
9		7.0	10	M	43	27	13 (30)	11	6	7	424
10		0.7	10	M	44	36	21 (48)	18	12	4	633
11		0.007	10	M	44	39	13 (29)	27	10	6	649
12	--	--	10	M	38	27	7 (18)	15	6	7	588

[a] Source: Toth et al., 1979

[b] TCPE = Trichlorophenoxy ethanol

[c] Carboxymethyl cellulose in groups 1-8, sunflower oil in groups 9-12.

[d] $p<1\%$

[e] $p<0.1\%$

In the oral study, (NTP, 1980a) groups of 50 male and 50 female rats received 2,3,7,8-TCDD by gavage in corn oil:acetone (9:1) 2 days/week for 104 weeks. The weekly dose of 2,3,7,8-TCDD was 0.01, 0.05 or 0.5 µg/kg bw. The male mice received identical oral doses of test compound on the above schedule, while female mice received weekly doses of 0.04, 0.2 and 2.0 µg/kg bw for 104 weeks. In both species an additional 3-week observation period followed treatment. Control groups for both rats and mice consisted of 75 untreated animals and 75 vehicle-treated animals of each sex. At death or termination of the study, gross and histopathologic examinations were performed on all major organs and gross lesions.

In this chronic study the only overt sign of toxicity in rats was a slightly lower body weight in the high dose male and female animals after 55 and 45 weeks of treatment, respectively. Survival among control and treated groups was not significantly different. Histologic examination revealed that toxic hepatitis was common in the high dosed animals, with the liver developing lipidosis and hydropic degeneration of the hepatocytes, and proliferation and fibrosis of the peripheral bile ducts. Histologic examination also revealed increased incidences of neoplastic lesions in both liver and thyroid. The incidence of hepatic neoplastic nodules or hepatocellular carcinomas was 0/74, 0/50, 0/50 and 3/50 (all neoplastic nodules) for males, and 5/75, 1/49, 3/50 and 15/49 for females in the control, low, medium and high dose groups, respectively. The incidence of hepatic neoplastic nodules in females was 5/75, 1/49, 3/50 and 12/49, respectively. In both males and females, the incidence of these combined lesions had a positive dose-related trend by the Cochran-Armitage test ($p=0.005$ and $p=0.001$ for males and females, respectively); however, the incidence of these tumors was significantly (p=0.001) higher than controls only in female rats in the high dose group. The incidence of hepatic neoplastic nodules in high-dose females was statistically significantly elevated (p=0.006) as compared to controls. In male rats, the incidence of thyroid follicular cell adenomas or carcinomas was 1/69, 5/48, 8/50 and 11/50 in the control, low, medium and high dose groups, respectively, and these tumor incidences showed a significant dose-related trend along with the incidences in the two higher doses being significantly (p=0.021 and 0.001, respectively) increased over controls. Incidence of other tumors, including thyroid follicular-cell adenomas, adrenal adenomas or carcinomas and subcutaneous adenomas in females, subcutaneous fibromas and cortical adenomas showed a dose-related trend or a significant increase over controls at the low or medium dose level, but did not meet the requirements for overall significance of 0.5 using the Bonferroni inequality criteria. The only tumors considered by the NTP to be related to exposure to 2,3,7,8-TCDD were the thyroid tumors in male rats and the liver tumors in female rats.

As observed in the rats, administration of 2,3,7,8-TCDD by gavage produced no overt signs of toxicity in mice with the only non-neoplastic histopathologic effect being toxic hepatitis. Neoplastic lesions that demonstrated both a significant dose-related trend and a greater incidence in the high dose animals included hepatocellular adenomas or carcinomas in both male and female mice and thyroid follicular adenomas in female mice. The incidence of liver tumors was 8/73, 9/49, 8/49 and 17/50 in males and 1/73, 2/50, 2/48 and 6/47 in females, while the incidence of thyroid tumors in females was 0/69, 3/50, 1/47 and 5/46 for the controls, low, medium and high dose groups, respectively. The incidence of histiocytic lymphomas and subcutaneous fibromas in female mice and alveolar/bronchiolar adenomas or carcinomas of the lung in male mice were significant by either the Cochran-Armitage test or the Fisher exact test but not both, and did not meet the Bonferroni inequality criteria for overall significance. Under test conditions it was concluded by the NTP (1980a) that 2,3,7,8-TCDD was carcinogenic to both male and female $B6C3F_1$ mice.

Cockerham et al. (1980) performed a field study on beach mice, Peromyscus polionotus, that inhabited an area which was heavily treated with the herbicide 2,4,5-T of which 2,3,7,8-TCDD was a contaminant. Analysis of the soil in the contaminated area revealed average 2,3,7,8-TCDD levels of 150 ppt at the surface. Measured levels of 2,3,7,8-TCDD in the liver of beach mice from the contaminated area was determined to be 1300 ppt in males and 960 ppt in females. Detection of 2,3,7,8-TCDD in the liver indicates that the compound was absorbed; however, since seeds in the area did not contain 2,3,7,8-TCDD it was believed that the animals ingested the compound from contaminated dust while grooming. In the 10 male and 5 female animals captured in the contaminated area, there were no histopathologic differences, including neoplastic lesions, observed in the liver as compared with 9 male and 6 female beach mice captured in a noncontaminated area. The only observed difference in the two groups of mice was a statistically significant (95% confidence) increase in liver to body weight ratios. The authors back-calculated from the 2,3,7,8-TCDD levels of the liver and estimated a daily 2,3,7,8-TCDD dose of 0.0012 µg/kg bw. It was noted that this exposure was much lower than the exposure used in laboratory studies to produce tumors.

2,3,7,8-TCDD (NTP, 1980b) has been tested in mice for tumorigenic potential by dermal application. This study was conducted under the NTP and the description of the chemicals used was the same as previously presented in the discussion of NTP (1980a).

Groups of 30 male and 30 female Swiss-Webster mice were treated with 100 μℓ of a solution of the test compound in acetone 3 times/week for 104 weeks. Groups of 45 animals were employed as vehicle controls and 2 groups of 15 animals were used as untreated controls. The concentration of 2,3,7,8-TCDD used resulted in a dose of 0.01 μg/application in male and 0.005 μg/application in female mice. Subchronic toxicity studies used to define the dose levels for the chronic bioassay indicated that all the doses used resulted in some liver damage but no increase in mortality. In the chronic study, animals were killed when moribund at the termination of the study and examined for gross tumors. Microscopic examinations of all major organs were also made.

In mice exposed dermally to 2,3,7,8-TCDD (NTP, 1980b), there was no treatment-related difference in body weight of either sex between exposed animals and control groups; however, male mice treated with 2,3,7,8-TCDD had a significant shortening of lifespan (i.e., 10/30, 7/45 and 3/30 survived to the end of the study in the untreated control, vehicle control and 2,3,7,8-TCDD-treated groups, respectively). Nontumorigenic hepatic lesions were observed in treated female mice, but not in treated male mice. The only tumors that were treatment related were integumentary system fibrosarcomas, with tumors developing on or near the site of application. The incidence of these tumors in male mice was 3/42 and 6/28, and in female mice the incidences were 2/41 and 8/27, respectively, for the vehicle control groups and the treated animals. Only the tumor incidence in female mice was statistically (p=0.007) greater than control values; however, life table analyses indicated that the time to tumor was shorter in both the male and female treated mice. The incidence of tumors in untreated and vehicle control groups was similar.

Using the mouse skin two-stage tumorigenesis model, 2,3,7,8-TCDD has been tested for initating and promoting activity. In this model, an initiator is a chemical that is applied to the skin for a very limited time under a dose schedule which will not result in tumor formation during the course of the study. Treatment with the initiator, however, is considered to have caused some irreversible change in the treated cells since subsequent repeated exposures to a promoting agent (a compound that does not produce tumors at the concentration tested after chronic exposure) results in the development of tumors. The results of these studies and similar studies assessing promotion of hepatocarcinogenesis are summarized in Table V-13. Also, in additional investigations, the effect of previous or simultaneous exposure to 2,3,7,8-TCDD on the tumor yield of known chemical carcinogens has been studied.

DiGiovanni et al. (1977) tested 2,3,7,8-TCDD, which was 98.6% pure, for initating activity on the skin of female CD-1 mice. Groups of 30 animals received an initial single dermal dose of 2,3,7,8-TCDD of 2 μg/mouse followed by twice weekly promotion with 5 μg of the known promoter 12-o-tetra-decanocyl-phorbol-13-acetate (TPA) for 32 weeks. The level of

TABLE V-13

Assessment of the Initiation and Promotion Activity of 2,3,7,8-TCDD in Laboratory Animals

Species/Strain	Sex	Number of Animals/Group	Initiator/Dose	Promoter/Dose	Tumor Type	% or Incidence of Animals With Tumors	Reference
Mice/CD-1	F	30	2,3,7,8-TCDD/ 2 μg/mouse	TPA/5 μg/application, 2 times/week for 32 weeks	dermal papilloma	14	DiGiovanni et al., 1977
	F	30	DMBA/ 2.56 μg/mouse	TPA/5 μg/application, 2 times/week for 32 weeks	dermal papilloma	63	
Mice/ Swiss-Webster	M	45	none	none	dermal papilloma	3/42	NTP, 1980c
	M	30	none	2,3,7,8-TCDD/0.001 μg/ application, 3 times/week for 104 weeks	dermal papilloma	6/28	
	M	30	DMBA/50 μg (single application)	2,3,7,8-TCDD/0.001 μg/ application, 3 times/week for 104 weeks	dermal papilloma	5/30	
Mice/ Swiss-Webster	F	45	none	none	dermal papilloma	2/41	
	F	30	none	2,3,7,8-TCDD/0.005 μg/ application, 3 times/week for 104 weeks	dermal papilloma	8/27	
	F	30	DMBA/50 μg (single application)	2,3,7,8-TCDD/0.005 μg/ application, 3 times/week for 104 weeks	dermal papilloma	8/29	
Mice/CD-1	F	30	none	2,3,7,8-TCDD/0.1 μg/ application, 2 times/week for 30 weeks	dermal papilloma	0.0%	Berry et al., 1978, 1979
	F	30	DMBA/200 nmol (single application)	2,3,7,8-TCDD/0.1 μg/ application, 2 times/week for 30 weeks	dermal papilloma	0.0%	
	F	30	DMBA/200 nmol (single application)	TPA/2 μg/application, 2 times/week for 30 weeks	dermal papilloma	92%	

TABLE V-13 (cont.)

Species/Strain	Sex	Number of Animals/Group	Initiator/Dose	Promoter/Dose	Tumor Type	% or Incidence of Animals With Tumors	Reference
Mice/HRS/J (hr/+)	F	20	DMBA/0.2 µmol (single application)	none	dermal papilloma	0/20	Poland et al., 1982
	F	20	none	2,3,7,8-TCDD/biweekly application of 50 ng/application for 8 weeks followed by 20 ng/application for 17 weeks	dermal papilloma	0/20	
	F	20	DMBA/0.2 µmol (single application)	2,3,7,8-TCDD/biweekly application of 50 ng/application for 8 weeks followed by 20 ng/application for 17 weeks.	dermal papilloma	0/20	
	F	20	DMBA/0.2 µmol (single application)	TPA/biweekly application of 2 µg/mouse	dermal papilloma	20/20	
Mice/HRS/J (hr/hr)	F	20	DMBA/0.2 µmol (single application)	none	dermal papilloma	1/20	
	F	20	none	2,3,7,8-TCDD/biweekly application of 50 ng/application for 8 weeks followed by 20 ng/application for 17 weeks	dermal papilloma	0/20	
	F	20	DMBA/0.2 µmol (single application	2,3,7,8-TCDD/biweekly application of 50 ng/application for 8 weeks followed by 20 ng/application for 17 weeks	dermal papilloma	15/19	
	F	20	DMBA/0.2 µmol (single application)	TPA/biweekly application of 2 µg/mouse	dermal papilloma	~70%	
Rats/ Charles River	F	4	DEN/10 mg/kg	none	hepatocellular carcinoma	0/4	Pitot et al., 1980
	F	4	none	2,3,7,8-TCDD/0.14 µg/kg twice a week for 28 weeks*	hepatocellular carcinoma	0/4	

TABLE V-13 (cont.)

Species/Strain	Sex	Number of Animals/Group	Initiator/Dose	Promoter/Dose	Tumor Type	% or Incidence of Animals With Tumors	Reference
Rats/ Charles River	F	5	none	2,3,7,8-TCDD/1.4 μg/kg twice a week for 28 weeks[a]	hepatocellular carcinoma	0/5	Pitot et al., 1980
	F	5	DEN/10 mg/kg	2,3,7,8-TCDD/0.14 μg/kg twice a week for 28 weeks[a]	hepatocellular carcinoma	0/5	
	F	7	DEN/10 mg/kg	2,3,7,8-TCDD/1.4 μg/kg twice a week for 28 weeks[a]	hepatocellular carcinoma	5/7	

[a] The 2,3,7,8-TCDD was administered by subcutaneous injection.

2,3,7,8-TCDD exposure was chosen from the ED_{50} for induction of aryl hydrocarbon hydroxylase activity, and this level resulted in the death of 1/3 of the animals before the end of the study. In the group initiated with 2,3,7,8- TCDD and promoted with TPA, there was a skin papilloma incidence of 14% with an average of 0.1 papillomas/mouse. This was in comparison with animals initiated with dimethylbenzathracene (DMBA), a potent tumor initiator, and promoted with TPA where the papilloma incidence was 63% with 2.2 papillomas/ mouse. The authors concluded that 2,3,7,8-TCDD was a weak tumor initiator; however, no vehicle control groups or TPA-treated-only control groups were included in the study for comparison.

The tumor promoting activity of 2,3,7,8-TCDD was investigated, along with the complete carcinogenicity in the NTP (1980b) bioassay. In the tumor promotion study, groups of 30 mice of each sex were initiated by a single dermal application of 50 μg of the tumor initiator (DMBA). A week after the initiation dose, the male mice received applications of 0.001 μg 3 times weekly and the female received 0.005 μg of 2,3,7,8-TCDD for 104 weeks. The skin tumor incidence in both male and female mice exposed to 2,3,7,8-TCDD plus DMA or the same level of 2,3,7,8-TCDD alone were nearly identical with respective tumor incidence in males of 5/30 and 6/28, and incidences in females of 8/29 and 8/27. Similar results were obtained by Berry et al. (1978, 1979) in which 30 female CD-1 mice were initiated with a single 200 nmol application of DMBA followed by twice weekly applications of 0.1 μg of 2,3,7,8-TCDD for 30 weeks. At the termination of promotion, there were no dermal papillomas in either the DMBA plus 2,3,7,8-TCDD group or the 2,3,7,8-TCDD group. In an additional group of mice initiated with DMBA followed by promotion twice a week with 2 μg of the known promoter

(TPA) there was, however, a 92% incidence of skin papillomas. The average number of tumors/mouse was 8.1. In the mouse skin two-stage tumorigenesis model, 2,3,7,8-TCDD did not demonstrate tumor promoting activity.

Poland et al. (1982) described studies which indicate that genetic differences in mice affect the tumor promoting capacity of 2,3,7,8-TCDD in the skin two-stage tumorigenesis model. Both 2,3,7,8-TCDD and TPA were compared for tumor promoting activity in DBA-initiated HRS/J mice that were either heterozygous (hr/+) or homozygous (hr/hr) for the recessive "hairless" trait. Promotion with biweekly applications of 2 μg of TPA for 25 weeks resulted in papilloma incidences of 100 and 70% in (hr/+) and (hr/hr) mice, respectively. Promotion of DMBA-initiated (hr/+) mice with 2,3,7,8-TCDD (50 ng/application for 8 weeks followed by 20 ng/application) did not result in the formation of tumors, while promotion of (hr/hr) mice resulted in both the same incidence and multiplicity of tumors as observed in TPA-promoted mice. With either DMBA or N-nitrosoguanidine (MNNG)-initiated (hr/hr) mice, the effective dose of 2,3,7,8-TCDD was ~100-fold less than TPA on molar basis. Histologic examination of the skin showed that TPA produced both acute inflammation and hyperplasia in (hr/+) and (hr/hr) mice, while 2,3,7,8-TCDD produced hyperplasia and hyperkeratosis only in (hr/hr) mice with no inflammatory response. The lack of a 2,3,7,8-TCDD-induced inflammatory response suggested to the authors that 2,3,7,8-TCDD-promoted skin papillomas in (hr/hr) mice by a mechanism different from TPA.

Pitot et al. (1980) investigated the ability of 2,3,7,8-TCDD to act as a promoter for the hepatocarcinogen diethylnitrosamine (DEN). Female Charles-River rats were given DEN at a single intragastric dose of 10 mg/kg during the time of liver regeneration after a 70% partial hepatectomy. Following this initiation with DEN, rats received twice weekly subcutaneous injections of either 0.14 or 1.4 μg of 2,3,7,8-TCDD/kg bw for 28 weeks. Additional groups of partially hepatectomized animals received only the initiation treatment or the promotion treatment. No tumors were observed in the rats given DEN or 2,3,7,8-TCDD alone, or in the animals exposed to DEN plus the low dose of 2,3,7,8-TCDD. In the rats exposed to DEN plus the high dose of 2,3,7,8-TCDD, however, the hepatocellular carcinoma incidence was 5/7. Similar high incidences, 8/10, of hepatocellular carcinomas were observed in rats exposed to DEN followed by dietary administration of phenobarbital, a promoter of liver carcinogenesis. Foci of cells with altered enzyme patterns indicative of preneoplastic lesions were identified in rats initiated with DEN and promoted with either dose level of 2,3,7,8-TCDD or phenobarbitol. Although this study is limited by the small size of the experimental groups, the authors concluded that 2,3,7,8-TCDD was a promoter for DEN-initiated hepatocarcinogenesis in rats.

Investigations have also been conducted on the effects of prior or simultaneous treatment with 2,3,7,8-TCDD on the subsequent development of skin tumors by chemical carcinogens. When 2,3,7,8-TCDD (0.1 μg) was administered simultaneously with DMBA (200 nmol) to the backs of CD-1 mice in a single initiation dose, the skin papilloma incidence following promotion with TPA was nearly the same as when DMBA alone was used as the initiator (DiGiovanni et al., 1977). Although simultaneous exposure to 2,3,7,8-TCDD and DMBA did not appreciably affect tumor yield, Berry et al. (1979) demonstrated a marked 93% decrease in the incidence of DMBA initiated tumors when CD-1 mice were pretreated 3 days before DMBA initiation with 1 μg/

mouse of 2,3,7,8-TCDD. The time of treatment with 2,3,7,8-TCDD in relation to initiation was shown to be critical in the antitumorigenic effect of 2,3,7,8-TCDD (Berry et al., 1979; Digiovanni et al., 1979b, 1980). Maximum tumor inhibition of between 86 and 95% occurred when pretreatment was between 1 and 5 days before initiation. If pretreatment was 10 days before DMBA initiation, the tumor yield was decreased by 78%, while 2,3,7,8-TCDD treatment 5 minutes before or 1 day after DMBA initiation had no effect on tumor yield. There was some indication of an inverse relationship between the pretreatment (3 days before DMBA initiation) dose of 2,3,7,8-TCDD and the incidence of tumors. Doses of 2,3,7,8-TCDD of 0.0, 0.01, 0.1 and 2 µg/mouse resulted in decreases in tumor yields, respectively, of 0, 83, 92 and 96% (DiGiovanni et al., 1979b). Also under similar experimental conditions Cohen et al. (1979) observed a 75% decrease in the incidence of skin tumors in Sencar mice pretreated with 1 µg of 2,3,7,8-TCDD 3 days before initiation by DMBA. Kouri et al. (1978) reported that i.p. injection of 1-100 mg 2,3,7,8-TCDD/kg preceded by MCA but not trioctanoin raised the carcinogenic index in B6 but not D2 mice. The term "carcinogenic index" was defined by Kouri et al. (1978) as percentage of tumor incidence 8 months after treatment divided by the average latency in days multiplied by 100.

DiGiovanni et al. (1980) investigated the antitumorigenic effect of 2,3,7,8-TCDD in CD-1 mice with chemical carcinogens other than DMBA. As observed with DMBA, exposure to 2,3,7,8-TCDD 3 days before initiation with either benzo(a)pyrene (BaP) or 3-MC resulted in a decrease in tumor yield as compared with acetone pretreated animals, while pretreatment with 2,3,7,8-TCDD 5 minutes before or 1 day after initiations was ineffective in changing the tumor yield. The maximum decrease in tumor production was 86 and 57%, respectively, for BaP and 3-MC initiated mice. A different temporal relationship was observed in the ability of 2,3,7,8-TCDD to inhibit tumor formation by BaP-diol-epoxide as compared with the previously studied polyaromatic hydrocarbons (PAH). When 2,3,7,8-TCDD was applied 3 days or 5 minutes before, or 1 day after initiation with BaP-diol-epoxide, decreases in tumor yield were 81.5, 49 and 39%, respectively. Examination of PAH metabolism in the skin of mice treated with 2,3,7,8-TCDD showed a 21-fold increase in aryl hydrocarbon hydroxylase (AHH) activity 72 hours after treatment (DiGiovanni et al., 1980). The in vitro metabolism of DMBA by dermal homogenates from 2,3,7,8-TCDD-treated mice indicated both qualitative and quantitative changes in metabolism (i.e., changes in both rates of metabolism and relative types of metabolites formed) (Cohen et al., 1979; DiGiovanni et al., 1979b; Berry et al., 1979). The similarity in the time frame of AHH induction and the antitumorigenic effect of pretreatment with 2,3,7,8-TCDD suggested that the antitumorigenic properties of 2,3,7,8-TCDD resulted from 2,3,7,8-TCDD-induced alteration in the metabolism of the initiating chemical. Although metabolic change was a possible mechanism for the inhibition of DMBA, 3-MC and BaP initiation, the ability of 2,3,7,8-TCDD to inhibit tumor yield when administered 1 day after initiation with BaP-diol-epoxide (which does not require metabolic activation) indicated to DiGiovanni et al. (1980) that more than one mechanism may participate in the anticarcinogenic effect of 2,3,7,8-TCDD.

Mutagenicity. Short-term in vitro test systems have been developed to assess the biologic, toxic and genotoxic effects of chemicals. These assays have proven to be useful indicators of potential activity of diverse industrial chemicals, a broad range of drugs and xenobiotics, carcinogens and crude environmental extracts. The most widely used short-term test system, the Ames test for bacterial mutagenesis, employs several strains of Salmonella typhimurium that are highly susceptible to the effects of mutagenic chemicals. Despite the obvious utility of the Ames test and related short-term assays, their predictive capabilities (i.e., the correlation between bacterial mutagenicity and carcinogenicity) have not been fully assessed (Bartsch et al., 1982).

Mutagenicity assays in microorganisms have been used to assess the genotoxic effects of 2,3,7,8-TCDD; however, the results of most of these assays have indicated little potential for mutagenic effects (Table V-14).

Hussain et al. (1972) exposed S. typhimurium histidine-dependent strains TA1530 and TA1532 in liquid suspension to 2,3,7,8-TCDD followed by plating into selective medium to observe reversion to prototypes. No increase in the reversion rate was observed with strain TA1530 at exposure levels of 1 and 10 μg/mℓ. These exposures resulted in cell survivals of 90 and <1%, respectively. In strain TA1532, increased reversion frequency was not observed at 2,3,7,8-TCDD concentrations of 2-3 μg/mℓ, which resulted in a 0-50% decrease in survival; however, at 2,3,7,8-TCDD levels that resulted in a 99% decrease in survival, there was an increased number of revertant colonies/surviving cells. The dose levels were not specified. The source of the 2,3,7,8-TCDD sample studied in this paper was the Food and Drug Administration, and its reported purity was 99%. Also, Seiler (1973) observed a positive mutagenic response in a spot test of 2,3,7,8-TCDD performed in the absence of a metabolic activation system. However, the purity of the sample studied was not provided. In tester strains G46 and TA1530,

TABLE V-14

The Results of Mutagenicity Assays for 2,3,7,8-TCDD in *Salmonella typhimurium*

Type of Assay	Strains of *Salmonella typhimurium*														Reference
	S-9	TA98	TA1530	TA1535	TA1537	TA1538	TA1532	TA1950	TA1975	TA1978	G46	TA100	TA1531	TA1534	
Spot test	+/-	NT	NT	0	0	0	0	NT	NT	NT	NT	NT	NT	NT	McCann, 1978
Plate incorporation	+/-	NT	NT	0	0	0	0	NT	NT	NT	NT	NT	NT	NT	McCann, 1978
Plate incorporation*	+/-	0	0	0	0	0	0	0	0	0	0	0	NT	NT	Gilbert et al., 1980
Fluctuation test	+/-	0	0	0	0	0	0	0	0	0	0	0	NT	NT	Gilbert et al., 1980
Spot test	-	NT	0	NT	NT	NT	+	NT	NT	NT	0	NT	QR	QR	Seiler, 1973
Plate incorporation	+	0	NT	0	0	0	NT	NT	NT	NT	NT	0	NT	NT	Geiger and Neal, 1981
Plate incorporation	-	NT	NT	NT	0	NT	NT	NT	NT	NT	NT	NT	NT	NT	Geiger and Neal, 1981
Suspension assay	-	NT	0	NT	NT	NT	+	NT	NT	NT	NT	NT	NT	NT	Hussain et al., 1972
Suspension assay	+/-	0	NT	0	0	NT	NT	NT	NT	NT	NT	0	NT	NT	Zeiger, 1983

*The assay was performed under both aerobic and anaerobic conditions.

NT = Not tested; QR = Questionable response; 0 = Negative response; + = Positive response the ratio of revertants/10^8 cells in the treated plates divided by spontaneous revertants/10^8 cells was <1. In strains TA1531 and TA1534, the ratio was between 1 and 2, which was considered a "doubtful" mutagenic response, while in strain TA1532, the ratio was >10. There was no mention of the 2,3,7,8-TCDD levels tested in this assay. The positive controls, diethylsulfate, 2-aminopurine and 2-aminofluorene, produced ratios of 2 to 5, <1 and 5 to 10, respectively, in strain TA1532. In both the study by Hussain et al. (1972) and the study by Seiler (1973), 2,3,7,8-TCDD produced a positive mutagenic response only in the S. typhimurium strain TA1532, which is sensitive to frameshift mutagens.

Hussain et al. (1972) also performed a mutagenicity test of 2,3,7,8-TCDD in two other microbial test systems. A positive response was observed in Escherichia coli Sd-4 as indicated by a reversion to streptomycin independence. In this assay, cells were treated in suspension for 1 hour with 2,3,7,8-TCDD at 0.5-4 μg/mℓ. The greatest mutation frequency (256 mutants x 10^{-8}, as compared with the control frequency of 2.2 mutants x 10^{-8}) occurred at a dose level of 2 μg/mℓ. The absolute number of colonies/plate was 7 for the control and 46 for the treated plate. The dose of 2 μg/mℓ caused an 89% decrease in cell survival. In the second test system, the ability of 2,3,7,8-TCDD to increase prophage induction in E. coli K-39 cells was examined. The vehicle control, DMSO, inhibited prophage induction as compared with the untreated controls, while the most effective dose level of 2,3,7,8-TCDD (0.5 μg/mℓ) resulted in an increased prophage induction as compared with the vehicle control but not as compared with the untreated controls. Hussain et al. (1972) concluded that 2,3,7,8-TCDD was capable of causing increases in the reverse mutation rate in _E. coli_ Sd-4 and that 2,3,7,8-TCDD had a weak ability to induce prophage in _E. coli_ K-39 cells.

The studies that followed these two early reports of Hussain et al. (1972) and Seiler (1973) failed to detect mutagenic activity of 2,3,7,8-TCDD in _S. typhimurium_. Wassom et al. (1978) cited a personal communication from McCann (1978), which reported that 2,3,7,8-TCDD was inactive in both the spot test and plate incorporation assay with _S. typhimurium_ strains TA1532, TA1535, TA1537 and TA1538. Doses and other experimental protocols were not mentioned except that the tests were performed both with and without metabolic activation. Gilbert et al. (1980) reported that 2,3,7,8-TCDD gave "substantially negative results" with _S. typhimurium_ strains TA98, TA100, TA1530, TA1535, TA1537, TA1538, G46, TA1532, TA1950, TA1975 and TA1978. Both the standard plate incorporation assay and the bacterial fluctuation test were used, and both were performed with and without S-9 prepared from the livers of Aroclor 1254 pretreated rats. In the plate incorporation assay, the test compound was tested at 1-2000 μg/plate under both aerobic and anaerobic conditions. Details were not provided for the fluctuation assay. It is difficult to assess possible reasons for the conflicting results between the earlier studies and these later mutagenicity assays, since information on experimental conditions was limited in the negative studies.

In an attempt to resolve the conflicting results and observe a mutagenic response, Geiger and Neal (1981) tested 2,3,7,8-TCDD in the standard plate incorporation assay using S-9 prepared from different sources. In order to maximize the amount of compound tested, dioxane, a better solvent for 2,3,7,8-TCDD than the commonly employed DMSO, was used. Even with the use of dioxane, the limited solubility of 2,3,7,8-TCDD allowed only 20 μg/plate to be tested, a dose that was shown to be non-toxic to the cells. The S-9 used in these assays was prepared from the livers of Aroclor 1254 pretreated male Sprague-Dawley rats and male Golden Syrian hamsters, and from 2,3,7,8-TCDD induced male hamsters. In all assays at 2,3,7,8-TCDD concentrations of 0.2, 2, 5 or 20 μg/plate, and regardless of the source of the S-9, there was no observed mutagenic response. In further attempts to duplicate the previous positive results, Geiger and Neal (1981) tested the same concentrations of 2,3,7,8-TCDD in strain TA1537, a more sensitive direct descendent of strain TA1532, for mutagenic activity in the absence of S-9. Again, no increase in the number of revertants was observed. In assays either with or without S-9, positive controls had predictable increases in the number of revertant colonies. The authors concluded that 2,3,7,8-TCDD was not active under the conditions of this assay; however, testing at higher concentrations may elicit a positive response. It was also noted that many other polychlorinated aromatic compounds are not mutagenic in the Ames test, even though there is positive evidence of carcinogenicity.

Mutagenic effects of 2,3,7,8-TCDD in yeast were observed by Bronzetti et al. (1983). Positive results for reversion and gene conversion were obtained in vitro and in the host-mediated assay. The in vitro experiments yielded small dose-related increases in trp$^+$ convertants and ilv$^+$ revertants. An S10 metabolic activation system was required. Exposure of the yeast to 2,3,7,8-TCDD at the highest level tested (10 μg/mℓ) resulted in 16% survival and yielded 4-fold increases in reversion and gene conversion.

In the host-mediated assay, male mice were exposed to 25 μg of 2,3,7,8-TCDD/kg (Bronzetti et al., 1983). After 5, 10, 20 or 30 days, 0.2 mℓ of a yeast culture (4×10^8 cells) was instilled retroorbitally. Four hours later, the liver and kidneys were removed and the yeast cells in these organs were assayed for mutagenic responses. Increases (4- to 6-fold) in reversion and gene conversion were observed in yeast cells obtained from the livers and kidneys. The toxic response of the animals to an exposure of 25 μg/kg was not described in this report. The positive results described in this paper suggest that 2,3,7,8-TCDD is mutagenic in yeast, but more definitive studies are needed before a firm conclusion can be drawn.

Hay (1982) has found that 2,3,7,8-TCDD dissolved in DMSO transformed baby hamster kidney cells (BHK) *in vitro*. The dioxin isomers 2,8-dichloro- and 1,3,7-trichlorodibenzo-*p*-dioxin also transformed BHK cells, but the response was weak. The unchlorinated dibenzo-*p*-dioxin and the fully chlorinated octachlorodibenzo-*p*-dioxin were both negative in the BHK assay (i.e., there was no cell transformation). More recently, Rogers et al. (1982) reported that 2,3,7,8-TCDD induced mutations in the excess thymidine, thioguanine and methotrexate selective systems in L5178Y mouse lymphoma cells in culture.

The National Toxicology Program (NTP) (Zeiger, 1983) provided data on 2,3,7,8-TCDD from four assay systems: the *S. typhimurium* (strains TA98, TA100, TA1535 and TA1537) histidine reversion assay, the sex-linked recessive lethal test in Drosophila, and cytogenetic studies (sister chromatid exchange and chromosome aberrations) in Chinese hamster ovary cells. Negative results were obtained in all of these assays. These studies cannot be evaluated, however, because the procedures used to obtain the data were not described.

In vitro reactions of 2,3,7,8-TCDD with bacteriophage QB RNA were evaluated by Kondorosi et al. (1973). Active RNA was purified from QB phage followed by incubation for 1 hour at 37°C with 0.0, 0.2, 2.0 or 4.0 μg/mℓ of 2,3,7,8-TCDD. At all concentrations tested, 2,3,7,8-TCDD had no effect on the transfectivity of QB RNA. Other compounds tested included the alkylating agents methyl, ethyl and isopropyl methane-sulfonate, and diethyl pyrocarbonate, all of which inactivated QB RNA under the same experimental conditions. The authors suggested that 2,3,7,8-TCDD inactivity in this assay indicated that 2,3,7,8-TCDD was an intercalating agent, and hence would require double stranded DNA in order to interact. The data presented in this study, however, were insufficient to support this conjecture.

In vivo binding of radiolabeled 2,3,7,8-TCDD to liver macromolecules was studied in Sprague-Dawley rats by Poland and Glover (1979). Both male and female animals were administered [1,6-^{3}H]2,3,7,8-TCDD i.p. at a dose of 7.5 μg/kg. This dose corresponded to a tritium level of 0.87 mCi/kg. The animals were killed 12, 48 and 168 hours after treatment, or 24 hours after treatment when the animals were pretreated with the enzyme inducers phenobarbital or unlabeled 2,3,7,8-TCDD. Following sacrifice, isolation of macromolecules, and removal of free labeled 2,3,7,8-TCDD, the amount of label bound to protein, RNA and DNA was determined. The greatest non-extractable binding of labeled 2,3,7,8-TCDD occurred to protein; however, the amount of label bound was small and only amounted to 0.03-0.1% of the total radioactivity administered. The total amount of label associated with RNA and DNA was, respectively, only 50 and 4 cpm above background. Time after exposure, sex or prior enzyme induction had no significant effect on 2,3,7,8-TCDD binding. As a result of the extremely low levels of radioactivity associated with RNA and DNA, it is uncertain whether 2,3,7,8-TCDD truly binds covalently to these macromolecules and, if so, whether there is any biological significance to this low level of apparent binding.

The effects of 2,3,7,8-TCDD exposure on the extent of chromosomal aberrations in the bone marrow of male rats were reported in an abstract by Green and Moreland (1975). In the initial experiment, no increase in chromosomal aberration was observed after five daily gavage treatments at a 2,3,7,8-TCDD dose of 10 μg/kg. In the second portion of this study, rats were exposed by a single intraperitoneal injection of 2,3,7,8-TCDD at 5, 10 or 15 μg/kg or a single gavage treatment at 20 μg/kg. The animals at the two highest exposure levels were killed 24 hours post-treatment, while the remaining animals were killed 29 days post-treatment. Again, no increase in chromosomal aberrations was observed, except in the positive control group exposed to triethylenemelamine.

In a later report, a small but significant increase in chromosomal aberrations was observed in the bone marrow cells of male and female Osborne-Mendel rats (Green et al., 1977). Bone marrow cells for cytogenetic analysis were obtained from Osborne-Mendel rats used in a range-finding study preliminary to a chronic bioassay (Green et al., 1977). The animals in groups of 8 males and 8 females received twice weekly intubations of 2,3,7,8-TCDD at respective doses of 0.25, 1.0, 2.0 and 4.0, or 0.25, 0.5, 2.0 and 4.0 µg/kg for 13 weeks. Because it was not required for the range-finding study, a control group was not included. Bone marrow cells were analyzed for abnormalities and cells in mitosis in the animals that survived to the end of the study (4-8 animals/group). The only significant increases in chromosomal aberrations in comparison with the low dose group were in males at 2 and 4 µg/kg and females at 4 µg/kg. The greatest incidence observed was 4.65% of the cells with chromosomal breaks in the high-dose males, and this was considered only weakly positive. The weak response, as well as the lack of data from control animals and the reported difficulty of obtaining cells from the high-dose animals as a result of 2,3,7,8-TCDD toxicity, makes the conclusion from this study that 2,3,7,8-TCDD produced chromosomal breaks tenuous.

A similar weak response was observed by Loprieno et al. (1982) in male and female CD-1 mice which received an i.p. injection of 2,3,7,8-TCDD at a dose of 10 µg/kg. At 96 hours post-treatment, there was a significant ($p<0.01$) increase in bone marrow cells with gaps and chromatid aberrations. When chromosomal aberrations were analyzed at 24 hours post-treatment, there was no significant change in the incidence of cells with aberrant chromosomes. The study was continued with a more extensive experiment using CD-COBS female rats. The rats were treated weekly by gavage (vehicle acetone-corn oil 1:6) at doses of 0, 0.01, 0.10 or 1.00 µg/kg for 45 weeks. Analysis of bone marrow cells for chromosomal aberrations 24 hours after the last treatment failed to detect any significant increases.

Czeizel and Kiraly (1976) reported an increased incidence ($p<0.001$) of chromatid-type and unstable chromosome aberrations in the peripheral lymphocytes of workers exposed to the herbicides 2,4,5-trichlorophenoxyethanol (2,4,5-TCPE) and Buminol. The 2,3,7,8-TCDD levels in the final product were <0.1 mg/kg; however, the exposure levels for individual workers were not available.

Mulcahy (1980) reported no increased incidences of chromosomal aberrations in the lymphocytes of 15 soldiers exposed to Agent Orange. The exposure was for 6-15 months and all subjects complained of symptoms, including skin eruptions, which they associated with Agent Orange. The analyses were performed with lymphocytes obtained ~10 years after the last exposure, and comparisons were made with eight subjects who had no history of exposure to 2,3,7,8-TCDD. Neither sister chromatid exchange nor structural aberrations including both gaps and breaks were increased. The authors note that the long time between exposure and analysis may have accounted for the negative results.

Also, both Reggiani (1980) and Mottura et al. (1981) have studied inhabitants in Seveso, Italy, exposed to 2,3,7,8-TCDD from an accident in a trichlorophenol manufacturing plant. Reggiani (1980) examined 4 adults and 13 children (3-13 years) for chromosomal aberrations within 2 weeks of the accident. These 17 individuals were examined to support claims of and determine extent of injury. Although burn-like skin lesions in these 17 individuals indicated chemical exposure, no increase in chromosomal aberrations was detected. The methods of performing the analyses and the actual number of aberrations detected were not described. Similar negative results were reported in an abstract by Mottura et al. (1981). In this study, subjects were chosen from the area of heavy contamination following the accident (acute high level exposure), from the working population of the plant (chronic low level exposure) and a nonexposed control population. The number of subjects in each group was not provided. The specimens were examined by three independent laboratories and no laboratory reported an increase in chromosomal aberrations, although there was a significant difference in the reported scores between laboratories. There was no information in this abstract on the extent of individual exposure or the length of time that elapsed between the accident and obtaining samples for analyses of chromosomal aberrations.

DiLernia et al. (1982) conducted additional studies on lymphocytes prepared in 1976 and 1979 from eight persons considered acutely exposed to 2,3,7,8-TCDD in the Seveso accident, eight ICMESA factory workers (considered chronically exposed), and 14 control subjects (eight had chromosome preparations made in 1976 and six in 1979). Cells were examined for average number of SAs (evidence for functional ribosomal genes), both on a cell basis and for the large acrocentric chromosomes (D group chromosomes). There was no change in the frequency of SAs on a per cell basis in any of the groups as compared to control values, nor in D group chromosomes from acutely exposed subjects examined immediately after the accident. There was, however, a decrease in the average frequency of SAs in group D chromosomes of acutely exposed subjects examined in 1977 and in ICMESA workers at both the 1976 and 1979 examinations. Although the biologic relevance of these observations has not yet been confirmed, DiLernia et al. (1982) observed a similar decrease in SAs after exposure of lymphocytes to x-irradiation. It was concluded that the decrease in SAs may have resulted from mutagenic damage to functional nucleolar organizing regions.

A limited number of initial studies on the mutagenicity of 2,3,7,8-TCDD in bacteria reported positive results in S. typhimurium strain TA1532 in the absence of a mammalian metabolic activation system (Hussain et al., 1972; Seiler, 1973). More recent attempts to repeat these results with strain TA1532 or related strains have failed (Geiger and Neal, 1981; Nebert et al., 1976; Gilbert et al., 1980; McCann, 1978). These authors have also reported no increase in mutation rate when 2,3,7,8-TCDD was tested in the presence of a mammalian metabolic activation system. In other in vitro assays, 2,3,7,8-TCDD has produced a positive response in reversion to streptomycin independence in E. coli Sd-4 cells and questionable positive response with prophage induction in E. coli K-39 cells (Hussain et al., 1972). Also, 2,3,7,8-TCDD has been reported to be mutagenic in the yeast S. cerevisiae in both the in vitro assay with S-10 and the host-mediated assay (Bronzetti et al., 1983). Rogers et al. (1982) have also reported positive mutagenicity results in the mouse lymphoma assay system. In the E. coli studies, the poor survival of the cells or the interference of the vehicle solvent, DMSO, with the assay makes the evaluation of the studies difficult. With the data available, it is not possible to resolve the conflicting reports on the mutagenic potential of 2,3,7,8-TCDD.

Overall, the data indicate little potential for the interaction of 2,3,7,8-TCDD with nucleic acids or the ability of 2,3,7,8-TCDD to produce chromosomal aberrations. Kondorosi et al. (1973) demonstrated that 2,3,7,8-TCDD did not react with RNA in vitro in the absence of a metabolic activation system. In vivo studies using radiolabeled 2,3,7,8-TCDD indicated some association of non-extractable label with RNA and DNA (Poland and Glover, 1979); however, the level of bound label was very low. Similar marginal data were available on the clastogenic effect of 2,3,7,8-TCDD. Although two *in vivo* studies in rats (Green and Moreland, 1975; Loprieno et al., 1982) failed to demonstrate any treatment-related chromosomal aberration, a second study by the same authors (Green et al., 1977) using a longer exposure period reported a small increase in the number of aberrations. A similar small increase was observed by Loprieno et al. (1982) following a single i.p. injection of 2,3,7,8-TCDD in mice. In humans exposed to 2,3,7,8-TCDD during the manufacture of 2,4,5-TCPE and Buminol, Czeizel and Kiraly (1976) reported an increase in the number of chromosomal aberrations, while no increase was detected in individuals exposed to 2,3,7,8-TCDD following an industrial accident in Seveso, Italy (Reggiani, 1980; Mottura et al., 1981). The studies of the clastogenic effect of 2,3,7,8-TCDD were presented with little or no experimental detail to assist in evaluating the merits of the reports. The data available are too limited to indicate whether 2,3,7,8-TCDD can interact with nucleic acids or produce chromosomal aberrations.

The differences among the results reported could be due to several factors, such as treatment protocols, solubility problems, purity of the samples tested and the high toxicity of 2,3,7,8-TCDD. This chemical may be a weak mutagen, but because it is very toxic, the dose range for detecting a positive genetic effect may be very narrow. Therefore, additional experimentation is necessary before any conclusive determination can be made. Suggested further testing includes the ability of 2,3,7,8-TCDD to induce forward mutations in mammalian cells in culture, additional yeast and bacterial studies and the sex-linked recessive lethal test in *Drosophila*.

Teratogenicity and Reproductive Toxicity. A number of investigators have studied the role of 2,3,7,8-TCDD contamination in 2,4,5-T-induced teratogenicity (Table V-15). Neubert and Dillmann (1972) investigated the teratogenicity of purified 2,3,7,8-TCDD, purified 2,4,5-T and samples of 2,4,5-T containing 0.05±0.02 ppm (0.05 mg/kg sample) or an undetermined amount of 2,3,7,8-TCDD. The ED_{50} for the production of cleft palate by 2,3,7,8-TCDD was determined to be 4.6 μg/kg bw.

Lamb et al. (1980) studied the effect of herbicides containing 2,3,7,8-TCDD on the reproductive behavior of male C57Bl/6 mice. 2,3,7,8-TCDD was mixed with 2,4-D and 2,4,5-T to simulate Agent Orange and added to the diet. At doses that resulted in hepatic and thymic toxicity, no significant changes were noted in mating frequency, average fertility, percentage implantation, number of resorption sites, percentage of fetal malformations, survival of offspring or neonatal development.

Rats are apparently less sensitive than mice to the teratogenic effects of 2,3,7,8-TCDD contaminated 2,4,5-T. Only skeletal anomalies (wavy ribs, fused sternum) indicative of fetal toxicity have been reported (Khera et al., 1971; Khera and McKinley, 1972). At doses <100 mg/kg bw/day of contaminated 2,4,5-T, the only skeletal effect appeared to be delayed ossification (Emerson et al., 1970, 1971; Sparschu et al., 1971b).

The teratogenicity of purified samples of 2,3,7,8-TCDD is presented in Table V-16. Courtney and Moore (1971) administered subcutaneous injections of 1 or 3 μg of purified 2,3,7,8-TCDD/kg bw/day to CD-1, DBA/2J and C57Bl/6J mice on days 6-15 of gestation. This treatment had no effect on

TABLE V-15

Studies on the Potential Teratogenic Effects of 2,3,7,8-TCDD Contaminated 2,4,5-T

Species/ Strain	Vehicle	Form of 2,4,5-T	TCDD Level	Daily Dose	Treatment Days	Observation Day	Maternal Response	Fetal Response	Reference
Mice/ NMRI	Rape-seed oil	acid	<0.02 ppm (Sample A)	8, 15, 30, 45, 60, 90, and 120 mg/kg	6-15	18	No toxic effects; decreased maternal weight at doses of 90 mg/kg and greater	Significant increases in the incidence of cleft palates at doses above 30 mg/kg (see text for additional details). Significantly decreased ($p<0.005$) fetal weight at all dose levels.	Neubert and Dillmann, 1972
	Rape-seed oil	acid	0.05±0.02 ppm (Sample B)	30, 60 and 90 mg/kg	6-15	18	No toxic effects; decreased maternal weight at 90 mg/kg	Increases in the incidence of cleft palate at 60 and 90 mg/kg; significant decrease in fetal weight ($p<0.005$) at all dose levels	
	Rape-seed oil	acid	NR (Sample C)	90 mg/kg	6-15	18	No toxic effects but decreased maternal weight	Increase in the incidence of cleft palate; significant ($p<0.005$) decrease in fetal weight	
	Rape-seed oil	butyl ester	NR	12 and 17 mg/kg	6-15	18	No toxic effects	Significant decrease in fetal weight but no effect on mortality; increase in the frequency of cleft palate similar to that seen with acid (see text)	
Mice/ NMRI	NR	acid	0.05±0.02 ppm	20, 35, 60, 90 and 130 mg/kg	6-15	NR	Toxic effects observed at 90 and 130 mg/kg	Increases in the percentage of resorptions and/or dead fetuses at 90 and 130 mg/kg; increases in the incidence of cleft palate and retardation of skeletal development at 35 mg/kg and above	Roll, 1971 (Taken from EPA, 1979a)
Mice/ CD-1	Corn oil: acetone (9:1)	acid	<0.05 ppm	115 mg/kg	10-15	18	No significant effect on weight gain or liver-to-body weight ratios	No effect on fetal mortality or fetal weight but an increase in the incidence of cleft palate	Courtney, 1977

TABLE V-15 (cont.)

Species/ Strain	Vehicle	Form of 2,4,5-T	TCDD Level	Daily Dose	Treatment Days	Observation Day	Maternal Response	Fetal Response	Reference
Mice/ C57Bl/6 or Mice/ AKR	Honey:water (1:1) or DMSO	acid	30 ppm	21.5, 46.4 and 113 mg/kg	6-14	18	NR	Significant ($p<0.01$) increases in the incidence of cleft palate in the high dose group and cystic kidney in both dose groups; increased fetal mortality also observed in the high dose group	Courtney et al., 1970a,b
Mice/ AKR	Honey:water (1:1)	acid	30 ppm	113 mg/kg	6-15	19	Increase in liver-to-body weight ratio	Significant ($p<0.05$) increases in the incidence of cleft palate and fetal mortality	Courtney et al., 1970a,b
Rats/ Sprague-Dawley (groups of 25 rats)	Gavage/ hydroxypropylmethylcellulose	acid	0.5 ppm	1, 3, 6, 12 or 24 mg/kg/ day	6-15	20	No effect on body weight and no observable signs of toxicity	A slight but statistically significant ($p<0.05$) decrease in implantations and litter size in lowest dose group only; no frank teratogenic effects based on a detailed examination of the control and 24 mg/kg dose group; the only effect noted was an increase in the incidence of 5th partially ossified sternebrae	Emerson et al., 1970, 1971 N.B. This appears to be a full publication of the abstract summary by Thompson et al., 1971
Rats/ Wistar	Gavage/ aqueous gelatin or corn oil	acid	<0.5 mg/kg	25, 50, 100 or 150 mg/kg/ day	6-15	22	Some maternal mortality and decreased body weight gain at 150 mg/kg; no signs of toxicity at 100 mg/kg or below	At 100 or 150 mg/kg, decreased fetal weight, increased fetal mortality and an increase in the incidence of skeletal anomalies; no significant effect at the two lower dose levels	Khera and McKinley, 1972; Khera et al., 1971
	Gavage/ aqueous gelatin or corn oil	butyl ester	<0.5 mg/kg	50 or 150 mg/kg/day	6-15	22	NR	No significant effect on fetal mortality, fetal weight, or the incidence of anomalies	Khera and McKinley, 1972; Khera et al., 1971

TABLE V-15 (cont.)

Species/ Strain	Vehicle	Form of 2,4,5-T	TCDD Level	Daily Dose	Treatment Days	Observation Day	Maternal Response	Fetal Response	Reference
Rats/ Holtzman	Gavage/ 1:1 solution of honey and water	acid	30 ppm	4.6, 10.0, and 46.4 mg/kg/day	10-15	20	NR	Significant ($p<0.01$) increases in fetal mortality at the 2 higher dose levels; dose-related increases in the percentage of abnormal fetuses per litter; a high incidence of cystic kidneys in treated groups	Courtney et al., 1970a,b
Rats/CD	Gavage/ 15% sucrose solution	acid	0.5 ppm	10.0, 21.5, 46.4 and 80.0 mg/kg/day	6-15	20	Reduced maternal weight gain at the 2 higher dose levels ($p<0.05$) and increased liver-to-body weight ratio at the highest dose level ($p<0.05$)	Increase in the incidence of kidney anomalies but no increase in cleft palate	Courtney and Moore, 1971
Rats/ Strain not specified	Gavage/ methocel	acid	0.5 ppm	50 mg/kg	6-15	NS	No effect on mortality or body weight gain	No significant effect on fetal mortality or fetal weight; a significant ($p<0.05$) increase in the incidence of delayed ossification	Sparschu et al., 1971b
	Gavage/ methocel	acid	0.5 ppm	100 mg/kg	6-10	NS	Increased mortality and decreased body weight gain	Increase in the incidence of delayed ossification and poorly ossified or malaligned sternabrae ($p<0.05$)	Sparschu et al., 1971b
Syrian hamsters/ _Mesocricetus auratus_	Gavage/ acetone, corn oil, and carboxymethyl cellulose in ratio of 1:5.8:10	acid	<0.1-4.5 ppm	20, 40, 80 and 100 mg/kg	6-10	14	NS	Dose-related increases in fetal mortality, gastrointestinal hemorrhages, and fetal abnormalities;	Collins et al., 1971

NS = Not specified; NR = Not reported

TABLE V-16

Studies on the Potential Teratogenic and Reproductive Effects of 2,3,7,8-TCDD

Species/Strain	Vehicle[a]	Compound	Daily Dose	Treatment Days	Observation Day	Maternal Response	Fetal Response	Reference
Mouse/CD-1 mouse/DBA/2J mouse/C57Bl/6J	DMSO[b]	2,3,7,8-TCDD	1, 3 μg/kg	6-15	17[c] or 18	increased liver/ body weight ratio	cleft palate, kidney anomalies[d]	Courtney and Moore, 1971
Mouse/C57Bl/6	Acetone:[a] corn oil (1:9)	2,3,7,8-TCDD	1, 3 μg/kg	10-13 or 10	18[c]	none reported	cleft palate, kidney anomalies[d]	Moore et al., 1973
Mouse/CD-1	DMSO[b] or corn oil	2,3,7,8-TCDD	25, 50, 100, 200, 400 μg/kg	7-16	18[e]	increased liver/ body weight ratio	cleft palate, hydronephrotic kidneys, hydrocephalus, open eyes, edema, petechiae	Courtney, 1976
Mouse/CF-1	corn oil[a]: acetone (98:2)	2,3,7,8-TCDD	0.001, 0.01, 0.1, 1.0, 3.0 μg/kg	6-15	18[c]	none reported	cleft palate, dilated renal pelvis	Smith et al., 1976
Mouse/NMRI	rape-seed[a] oil	2,3,7,8-TCDD	0.3, 3.0, 4.5, 9.0 μg/kg	6-15	18	no effect observed	fetocidal at the high dose, cleft palate at doses at or above 3 μg/kg	Neubert and Dillmann, 1972
Rat/CD	DMSO[b]	2,3,7,8-TCDD	0, 0.5, 2.0 μg/kg	6-15, 9 and 10, or 13 and 14	20[c]	none reported	kidney malformations at both dose levels	Courtney and Moore, 1971
Rat/Sprague-Dawley	corn oil[a]/ acetone	2,3,7,8-TCDD	0, 0.03, 0.125, 0.5, 2.0 and 8.0 μg/kg	6-15	20[c]	vaginal hemorrhage at 2.0 and 8.0 μg/kg	intestinal hemorrhage at 0.125 and 0.5 μg/kg, fetal death of higher doses, subcutaneous edema	Sparschu et al., 1971a

TABLE V-16 (cont.)

Species/Strain	Vehicle[a]	Compound	Daily Dose	Treatment Days	Observation Day	Maternal Response	Fetal Response	Reference
Rat/Wistar	corn oil[a]/ anisole	2,3,7,8-TCDD	0.0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16 μg/kg	6-15	22	maternal toxicity observed at or above 1 μg/kg	increased fetal death observed at or above 1 μg/kg, subcutaneous edema and hemorrhages in the 0.25-2.0 μg/kg groups	Khera and Ruddick, 1973
Rat/Sprague-Dawley	corn oil[a]/ acetone (9:1)	2,3,7,8-TCDD	0.0, 0.125, 0.5, 2.0 μg/kg	1-3	21	decrease in body weight gain in the high dose group	decreased fetal weight in the 0.5 and 2 μg/kg group, cystic kidneys and dilated renal pelvis occured in the 2 μg/kg group	Giavini et al., 1982a
Rat/Sprague-Dawley	diet	2,3,7,8-TCDD	0.001, 0.01 and 0.1 μg/kg[f]	throughout gestation	postparturition	low fertility at 0.01 and 0.1 μg/kg, decreased body weight at 0.01 and 0.1 μg/kg, dilated renal pelvis	low survival at 0.01 and 0.1 μg/kg, decreased body weight at 0.01, slight dilated renal pelvis at 0.001 μg/kg	Murray et al., 1979
Rabbit/ New Zealand	corn oil[a]/ acetone (9:1)	2,3,7,8-TCDD	0.0, 0.1, 0.25, 0.5 and 1 μg/kg	6-15	28	maternal toxicity at doses of 0.25 μg/kg and above	increases in extra ribs and total soft tissue anomalies	Giavini et al., 1982b
Monkey/rhesus	diet	2,3,7,8-TCDD	8.6 pg/kg/day	7 months before and during gestation	at term	6/8 conceived, normal serum estradiol and progesterone	3/8 normal births	Allen et al., 1979
Monkey/rhesus	diet	2,3,7,8-TCDD	55.7 pg/kg/day	7 months before and during gestation	at term	3/8 conceived, decreased serum estradiol and progesterone	1/8 normal births	Allen et al., 1979

[a] Administration was by gavage.

[b] Administration was by subcutaneous injection.

[c] First day of gestation designated day zero.

[d] Kidney anomalies were not specifically defined.

[e] First day of gestation designated day one.

[f] The high dose level (0.1 μg/kg/day) was discontinued because of very low fertility in adults.

fetal mortality; however, cleft palate and unspecified kidney anomalies were found at all dose levels in all strains, with C57Bl/6J being the most sensitive strain.

Moore et al. (1973) administered oral doses of 2,3,7,8-TCDD (1 or 3 μg/kg/day) to C57Bl/6 mice by gavage on days 10-13 of gestation. Cleft palate and hydronephrosis were observed in both dose groups. These kidney lesions were apparently reversible, since very few litters (1/14) that were cross-nursed on control mothers had pups with kidney abnormalities. In contrast, kidney lesions were found in 4 of 14 control litters that were nursed on 2,3,7,8-TCDD treated mice.

Neubert and Dillman (1972) and Neubert et al. (1973) administered daily oral doses of 0.3, 3.0, 4.5 or 9.0 μg/kg bw to NMRI mice on days 6-15 of gestation. Extensive resorption (6 of 9 litters completely resorbed) occurred in the high-dose group. Cleft palate, but not kidney abnormalities, were observed at a dose of 3.0 μg/kg bw/day or higher, with 0.3 μg/kg bw/day being the NOAEL in this study. A single dose of 45 μg/kg bw could produce cleft palate if given before day 13 of gestation, with maximum incidences occurring when the dose was given on day 8 or 11.

Courtney (1976) compared the effectiveness of oral and subcutaneous administration in CD-1 mice. Subcutaneous administration was found to produce a greater teratogenic response at a lower dose than did oral administration.

Smith et al. (1976) determined the minimum effective oral dose (MED) for producing teratogenic effects in CF-1 mice to be 1 μg 2,3,7,8-TCDD/kg bw/day. The NOAEL in this study was 0.1 μg/kg bw/day.

Courtney and Moore (1971) determined the teratogenic potential of subcutaneously injected 2,3,7,8-TCDD (0.5 or 2 μg/kg bw/day) in CD rats. The compound was administered in dimethylsulfoxide on days 6-15, 9-10 or 13-14 of gestation. The only developmental anomalies observed were kidney malformations in all dose groups. Six hemorrhagic gastrointestinal tracts were found; however, this was considered a primary fetotoxic effect and not a malformation.

Sparschu et al. (1971a) administered 0.03, 0.125, 0.5, 2.0 or 8.0 μg 2,3,7,8-TCDD/kg/day by gavage to Sprague-Dawley rats on days 6-15 of gestation. A number of dose-related fetotoxic effects were found, including edema, increased numbers of resorptions, numbers of dead fetuses and intestinal hemorrhage. No teratogenic effects were reported in the surviving fetuses.

Khera and Ruddick (1973) intubated groups of 7-15 pregnant Wistar rats with 0.125, 0.25, 0.5, 1, 2, 4, 8 or 16 μg 2,3,7,8-TCDD/kg bw/day on days 6-15 of gestation. Severe fetotoxic effects were observed at doses of 1 μg/kg bw/day or higher, with no live fetuses found in the groups exposed to 4, 8 or 16 μg/kg bw/day. No anomalies were observed in the group receiving 0.125 μg/kg bw/day. In the intermediate dose groups, 0.25-2.0 μg/kg bw/day, a number of anomalies, including subcutaneous edema of the head and neck and hemorrhages in the intestine, brain, and subcutaneous tissue, were observed. When the dams were allowed to litter and wean the pups, none of the pups in groups receiving >1 μg/kg bw/day survived until weaning. Fostering pups from dams exposed to 1 μg/kg bw/day to control dams did not appreciably increase survival (36/42 died).

Giavini et al. (1982a, 1983) administered 0, 0.125, 0.5 or 2.0 μg 2,3,7,8-TCDD/kg/day by gavage to Sprague-Dawley rats on days 1-3 days of gestation or to female CRCD rats daily for 2 weeks before mating. In the groups dosed on days 1-3 of gestation, fetal weight was significantly decreased in the 0.5 and 2.0 μg/kg bw/day groups, but no statistically significant increases in malformations were noted. When adult female rats were treated for 2 weeks before mating, an increased number of cystic kidneys and dilated renal pelvis were observed in the pups in the high dose group. In these studies, 0.125 μg/kg bw/day was the NOEL for both maternal toxicity and adverse effects on the fetus.

The reproductive effects of 2,3,7,8-TCDD were also studied in a 3-generation study using Sprague-Dawley rats (Murray et al., 1979). Throughout the study, animals were continuously maintained on diets providing doses of 0, 0.001, 0.01 or 0.1 μg 2,3,7,8-TCDD/kg/day. The parental group (f_0) was maintained for 90 days on the test diets prior to mating. The f_0 rats were mated twice, producing the filial generations (f_{1A} and f_{1B}). Selected f_{1B} and f_2 rats were mated at ~130 days of age to produce the f_2 and f_3 litters, respectively. In later generations, the high dose group (0.1 μg 2,3,7,8-TCDD/kg/day) was discontinued because few offspring were produced in this group. At the intermediate dose (0.01 μg/kg/day), 2,3,7,8-TCDD caused lower body weight in exposed rats of both sexes (f_1 and f_2). At the low dose, no toxic effects were discerned.

Fertility was greatly reduced in the f_0 generation exposed to 0.1 μg 2,3,7,8-TCDD/kg/day. At 0.01 μg 2,3,7,8-TCDD/kg/day, fertility was significantly ($P<0.05$) reduced in the f_1 and f_2 rats. Fertility in rats (of any generation) exposed to 0.001 μg 2,3,7,8-TCDD/kg/day was not different from that of control rats. Decreases in litter size were noted in the f_{1A} group exposed to 0.1 μg/kg/day and the f_2 and f_3 litters exposed at 0.01 μg/kg/day. Statistically significant decreases in fetal survival throughout gestation were noted in f_2 and f_3 litters of the 0.01 μg 2,3,7,8-TCDD/kg/day exposed dams. At 0.001 μg 2,3,7,8-TCDD/kg/day, a decreased gestational survival was reported for the f_2 litters, but not for other generations. Decreased neonatal survival was noted among f_{1A} and f_2 pups exposed to 0.01 μg 2,3,7,8-TCDD/kg/day, but not among f_{1B} or f_3 pups. Postnatal body weights of the f_2 and f_3 litters at 0.01 μg 2,3,7,8-TCDD/kg/day were significantly depressed. At the low dose (0.001 μg 2,3,7,8-TCDD/kg/day), necropsy of 21-day-old pups revealed a statistically significant ($P<0.05$) increase in dilated renal pelvis in the f_1 generation. Subsequent generations at this dose level or any at the intermediate dose (0.01 μg 2,3,7,8-TCDD/kg/day) did not have a significant increase in this abnormality. Significantly decreased thymus weight and increased liver weight were reported in the f_3 generation, but not in the f_1 generation (f_2 generation data not obtained) of the intermediate dose group. Murray et al. (1979) concluded that 2,3,7,8-TCDD ingested at 0.01 or 0.1 μg/kg/day impaired reproduction among rats, and NOAELs were associated with 0.001 μg 2,3,7,8-TCDD/kg/day.

Nisbet and Paxton (1982) reevaluated the primary data of Murray et al. (1979) using different statistical methods. From this reevaluation it was concluded that 2,3,7,8-TCDD significantly reduced the gestational index, decreased fetal weight, and increased liver to body weight ratios and the incidence of dilated renal pelvis in both lower dose groups. Nisbet and Paxton (1982) concluded that the dose of 0.001 μg/kg/day was not a NOAEL in this study. The FIFRA Scientific Advisory Panel has also reviewed the data from this three generation study and concluded that the effects observed at the 0.001 μg/kg dose were not consistent enough between the different generations to consider them treatment-related (U.S. EPA, 1979b). Although the panel considered the data suggestive of an embryotoxic effect, they concluded that 0.001 μg/kg represented a NOEL. Subsequently, EPA did further evaluation of Murray et al. (1979) data and arrived at a conclusion that 0.001 μg/kg represents a LOAEL (U.S. EPA, 1984a).

Berry et al. (1976, 1977) treated Sprague-Dawley rats on day 17 of gestation with 0.2, 0.5, 2.5 or 6 μg 2,3,7,8-TCDD/kg bw intraperitoneally. Maximal induction of fetal hepatic aryl hydrocarbon hydroxylase (AHH) activity (measured by fluorometric methods) and N-hydroxylation of FAA (measured by autoradiography) was observed at a dose of 2.5 μg/kg bw. At lower doses these effects were not observed in either the fetus or the dams. Electron microscopic examination revealed cellular necrosis, increased glycogen and rough endoplasmic reticulum and swollen mitochondria in the liver at dose levels that induced AHH activity. Induction of epoxide hydratase was also observed in the lungs (ratio 2,3,7,8-TCDD/control=2.85), kidney (ratio 2,3,7,8-TCDD/control=1.06) and skin (ratio 2,3,7,8-TCDD/ control=2.62).

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Lucier and McDaniel (1979) intubated CD rats with 0 or 3 μg 2,3,7,8-TCDD/kg on days 5, 10 or 16 of gestation. They measured the activity of fetal and newborn hepatic microsomal benzo[a]pyrene hydroxylase and p-nitrophenol glucuronide formation on gestation day 21, postnatal day 8 and postnatal day 21. The BaP hydroxylase activity of controls on gestation day 21, treated rats on gestation day 21, controls on postnatal day 8, treated rats on postnatal day 8, controls on postnatal day 21 and treated rats on postnatal day 21 was 0.003, 0.032 ($p<0.01$), 0.214, 0.719 ($p<0.01$), 0.224 and 0.863 ($p<0.01$) nmol/min/mg, respectively. The rate of p-nitrophenyl glucuronide formation at the above intervals was 13.4, 14.4, 28.4, 99.5 ($p<0.01$), 23.1 and 164.2 ($p<0.01$) nmol/min/mg, respectively.

Giavini et al. (1982b) administered 0.1, 0.25, 0.5 or 1 μg 2,3,7,8-TCDD/kg bw/day to groups of 10-15 New Zealand rabbits by gavage on days 6-15 of gestation. Maternal toxicity was observed at doses of 0.25, 0.5 or 1.0 μg/kg bw/day. There were increases in abortions and resorptions at doses of 0.25 or 0.5 μg/kg bw/day, with no live fetuses found in the 1.0 μg/kg bw/day dose groups. Extra ribs were found in all dose groups. Hydronephrosis was a common finding in all groups, but the increase in treated groups over control values was not statistically significant. 2,3,7,8-TCDD also induced fetal liver microsomal enzymes in New Zealand rabbits (Norman et al., 1978). The BaP hydroxylase activity of the liver microsomal fraction of adult males and newborn pups was determined following subcutaneous injection of 30 nmol/kg into adult animals. Dams received treatment on the 24th day of gestation. The benzo[a]pyrene hydroxylase activity of newborn controls, adult controls, treated newborns and treated adults was 0.3, 1.8, 1.6 and 3.7 nmol/mg protein, respectively.

Allen et al. (1979) fed adult female rhesus monkeys on diets containing 50 or 500 ppt (50 or 500 ng/kg diet) 2,3,7,8-TCDD for 7 months. The animals consumed a total dose of 1.8 and 11.7 μg 2,3,7,8-TCDD, respectively. The animals were bred at 7 months of treatment resulting in pregnancy in 6 of 8 females in the low-dose group and 3 of 8 in the high-dose group. They were continued on treatment during pregnancy. In both groups, two-thirds of the pregnancies ended in spontaneous abortions. There were no reported malformations in the three surviving infants. All of the controls (one group of 8 and another of unspecified size) conceived and gave birth to normal offpsring. McNulty (1978) reported a dose-related increase in spontaneous abortions in rhesus monkeys given oral doses of 0.0, 0.2, 1 or 5 μg/kg bw 3 times/week for 3 weeks starting ~20 days preconception. The group sizes (2-4 animals) were too small for adequate statistical analysis.

Summary

There are wide variations in the species sensitivity to the acute toxicity of 2,3,7,8-TCDD. LD_{50}s range from 0.6 μg/kg bw for the male guinea pig to >5000 μg/kg bw for the male hamster (Schwetz et al., 1973; Olson et al., 1980b; Henck et al., 1981). The toxic manifestations seem to be the same whether the compound is given as a single oral dose or as a limited number of multiple treatments, with death occurring from 5-45 days post-treatment. Lethal exposures result in weight loss, often described as "wasting away," and thymic atrophy. In some species, particularly rats and mice, extensive liver damage is observed (Gupta et al., 1973). In general, no specific cause of death has been identified, although extensive hemorrhaging has been implicated in mice (Vos et al., 1974).

In rats and mice, single high doses produce liver necrosis (Jones and Butler, 1974), while lower doses produce fatty changes and proliferation of the endoplasmic reticulum (Fowler et al., 1973). Other effects seen in some species include induction of microsomal enzymes, degeneration of plasma membranes with loss of ATPase activity, a decreased ability to excrete some xenobiotics in the bile, porphyria, altered gastrointestinal absorption of some nutrients and decreased blood cellularity.

2,3,7,8-TCDD is an immunotoxin, predominately affecting cell-mediated immunity. Hypersensitivity, adverse effects on the thymus and increased sensitivity to antigens have demonstrated the immunotoxic potential of 2,3,7,8-TCDD.

In rats and mice, the liver appears to be the most sensitive organ following chronic or subchronic exposure. Hepatotoxicity develops following a long induction period and the changes persist for long periods following the termination of exposure (King and Roesler, 1974; Goldstein et al., 1982b).

In the subchronic studies reviewed in this report, the NOAEL of 0.01 μg/kg bw/day (Kociba et al., 1976) and 0.07 μg/kg bw/day (NTP, 1980a) have been reported for rats. A NOAEL of 0.29 μg/kg bw/day was evident for female mice and a LOEL of 0.14 μg/kg bw/day for male mice in the NTP (1980a) study. A NOAEL of 0.001 μg/kg bw/day, a NOAEL of 0.05 μg/kg bw/day, and a frank effect level (FEL) of 0.1 μg/kg bw/day have been reported for rats following chronic exposure (Kociba et al., 1978a,b, 1979; NTP, 1980a). Toth et al. (1978, 1979) observed toxic effects in mice at doses as low as 0.007 μg/kg bw/week.

In a preliminary study by Van Miller (1977a,b), 2,3,7,8-TCDD was tested for carcinogenicity following oral administration to rats. Increases in the incidence of total tumors was observed in some groups; however, the group sizes, ~10 animals/group, were too small for an assessment of a treatment-related response. In a second, more extensive study by Kociba et al. (1978a) a positive carcinogenic response was detected. In this study the estimated intake of 2,3,7,8-TCDD from the diet was 0.0, 0.001, 0.01 and 0.1 μg/kg/day. In the high dose group, both male and female animals had significant increases in site specific tumors. The target organs and tumor types in male animals were squamous cell carcinomas of the tongue and hard palate, and adenomas of the adrenal cortex; in female animals the target organs and tumor types were hepatocellular carcinomas, squamous cell carcinomas of the tongue and of the lung. The data demonstrate that dietary exposure to 2,3,7,8-TCDD at levels that produce a daily dose of 0.1 μg/kg produce increased tumor incidences in both male and female rats. Though the increase was not significant, these tumor types were also found in lower dose groups.

Under the National Toxicology Program, 2,3,7,8-TCDD was tested for carcinogenicity in rats following administration by gavage (NTP, 1980a). Both male and female animals were exposed to weekly doses of 0.0, 0.01, 0.05 and 5 μg/kg bw. The only tumors that appeared to be treatment-related were follicular cell adenomas or carcinomas of the thyroid in male animals, and neoplastic nodules or hepatocellular carcinomas of the liver in female animals. The incidence of these tumors was significantly greater than control in the high dose groups and the incidence of both tumors showed a positive dose-related trend. Under the conditions of this assay, 2,3,7,8-TCDD was concluded to be carcinogenic in both male and female rats.

Further studies in mice exposed by gavage have provided support for the carcinogenicity of 2,3,7,8-TCDD. Toth et al. (1979) exposed male mice to 2,3,7,8-TCDD at doses of 0.0, 0.007, 0.7 and 7.0 μg/kg week in a study to determine whether 2,4,5-TCPE, its contaminant 2,3,7,8-TCDD, or both were carcinogens. At the 0.7 μg/kg/week level there was a significantly increased incidence of liver tumors. Liver tumors were not significantly increased in the high dose group; however, early mortality in this group from high doses may have precluded observing late developing tumors. Similarly increased incidences of liver tumors were observed in the NTP (1980a) study in the high dose male mice exposed to 0.5 μg/kg/week and in the high dose female mice exposed to 2 μg/kg/week of 2,3,7,8-TCDD by gavage. Female mice also had an increased incidence of follicular-cell adenomas of the thyroid. In both studies, 2,3,7,8-TCDD was carcinogenic to mice with effective doses ranging between 0.5 and 2 μg/kg/day depending on sex and the individual study.

The mouse skin two-stage tumorigenicity model has also been used to test the carcinogenic potential of 2,3,7,8-TCDD. Following long-term dermal application 3 times/week of 2,3,7,8-TCDD at levels of 0.01 and 0.005 μg/application to male and female mice, respectively, there was an increased incidence of skin tumors only in female mice (NTP, 1980b). Along with the indication that 2,3,7,8-TCDD was a complete carcinogen in this system, DiGiovanni et al. (1977) reported that 2,3,7,8-TCDD was also a tumor initiator in mouse skin. The ability of 2,3,7,8-TCDD to initiate, however, has yet to be confirmed since appropriate vehicle and promotion-only control groups were not included. Attempts to demonstrate tumor promoting activity with 2,3,7,8-TCDD on mouse skin have produced negative results in some assays (NTP, 1980b; Berry et al., 1978, 1979); however, Poland et al. (1982) reported that 2,3,7,8-TCDD was a tumor promoter when tested on the skin of mice homozygous for the "hairless" trait but not in mice heterozygous for this recessive trait. Pitot et al. (1980) also reported that 2,3,7,8-TCDD was a promoter for DEN-initiated hepatocarcinogenesis in rats following parenteral administration of the compounds. On mouse skin, 2,3,7,8-TCDD was a complete carcinogen and possibly a tumor initiator, while no tumor promoting activity could be attributed to 2,3,7,8-TCDD in the assays. In rat liver initiated with DEN, 2,3,7,8-TCDD was a tumor promoter.

In studies of the interaction of 2,3,7,8-TCDD with other chemical carcinogens, Kouri et al. (1978) reported that 2,3,7,8-TCDD was a cocarcinogen with 3-MC when administered by subcutaneous injection. In the mouse skin bioassay, initiation with simultaneous administration of 2,3,7,8-TCDD and DMBA, however, did not affect tumor yield (DiGiovanni et al., 1977). Similarly, no effect was observed when 2,3,7,8-TCDD was administered either immediately before (5 minutes) or 1 day after DMBA initiation (Berry et al., 1979; DiGiovanni et al., 1977, 1979a; Cohen et al., 1979). When treatment with 2,3,7,8-TCDD occurred 1-10 days before DMBA initiation, 2,3,7,8-TCDD demonstrated a potent anticarcinogenic action. Although 1-5 days prior exposure to 2,3,7,8-TCDD inhibited tumor initiation by BaP, 3-MC and BaP-diol-epoxide, the tumor initiating ability of the latter compound was also inhibited when 2,3,7,8-TCDD exposure occurred either 5 minutes before or 1 day after initiation (DiGiovanni et al., 1980). The increased AHH activity resulting from 2,3,7,8-TCDD exposure may account for the anticarcinogenic activity by altering the metabolism of the initiating compound; however, DiGiovanni et al. (1980) suggest that the inhibition of the initiating activity of BaP-diol-epoxide 1 day after initiation indicates that more than one mechanism participates in the anticarcinogenic activity of 2,3,7,8-TCDD.

Early reports indicated that 2,3,7,8-TCDD was mutagenic in *S. typhimurium* strain TA1532 (Hussain et al., 1972; Seiler, 1973); however, later attempts to confirm these results have been unsuccessful (Geiger and Neal, 1981; Nebert et al., 1976; Gilbert et al., 1980; McCann, 1978). 2,3,7,8-TCDD has been reported to be mutagenic to *E. coli* *in vitro* (Hussain et al., 1972) and to *S. cerevisiae* *in vitro*, and in the host-mediated assay (Bronzetti et al., 1980). Covalent interactions with nucleic acids are minimal if they occur at all (Kondorosi et al., 1973; Poland and Glover, 1979). Only marginal effects have been observed on the incidence of chromosomal aberrations *in vivo* (Green and Moreland, 1975; Green et al., 1977).

2,3,7,8-TCDD has been demonstrated to be teratogenic in all strains of mice tested. The most common malformations observed are cleft palate and kidney anomalies; however, other malformations have been observed occasionally. With a MED of 1 μg/kg/day, 2,3,7,8-TCDD is the most potent teratogen known. At higher doses, 2,3,7,8-TCDD has a marked fetotoxic effect, as measured by decreased fetal weight and increased fetal toxicity. Hemorrhagic GI tract has been associated with 2,3,7,8-TCDD fetal toxicity.

In rats, it has also been consistently observed that 2,3,7,8-TCDD produced teratogenic and fetotoxic responses in all strains tested. In this species, the most common fetal anomalies observed were edema, hemorrhage and malformation of the kidney with effects observed at doses of $\geq$0.01 μg/kg/day. In addition, there is some evidence that 2,3,7,8-TCDD can induce microsomal enzymes in the fetus exposed _in_ _utero_, and this induction is accompanied by damage to the fine structure of the liver cell; however, other reports indicate that enzyme induction occurs only after birth following exposure to 2,3,7,8-TCDD through the mother's milk. As in mice, hemorrhagic GI tracts have been observed in rat fetuses exposed _in_ _utero_ to 2,3,7,8-TCDD.

Rabbits and monkeys are also susceptible to the fetotoxic effects of 2,3,7,8-TCDD; however, the studies of these species have been too limited to clearly demonstrate a teratogenic response or define a threshold dose for fetotoxicity.

VI. HEALTH EFFECTS IN HUMANS

Clinical Case Studies

Acute exposure to 2,3,7,8-TCDD results in nausea and vomiting, headache, and irritation of the eyes, skin and respiratory tract. The initial skin reaction is a cutaneous reaction resembling a chemical burn, followed several days to weeks after by chloracne (Taylor, 1979). Chloracne, the typical human dermal reaction to 2,3,7,8-TCDD, is a cutaneous eruption of comedones, cysts, and in severe cases, pustules. These usually occur on the face and shoulders as a result of squamous metaplasia of the dermal glands (Crow, 1978; Passi et al., 1981). Most of the documented acute exposures to 2,3,7,8-TCDD have been the result of chemical industry accidents involving 2,4,5-trichlorophenoxyacetic acid, which is contaminated with 2,3,7,8-TCDD.

According to Holmstedt (1980), the first cases of chloracne associated with exposure to dioxins occurred following an explosion in a chemical plant producing 2,4,5-T in 1949. Zack and Suskind (1980) recorded nausea, headaches, fatigue, muscular aches and pains, and chloracne as the frequent complaints among the 228 workers exposed. Chemical tests revealed elevated lipid levels and prolonged prothrombin times. Residual chloracne, peripheral neuropathy, fatigue and severe aches and pains persisted for up to 2 years.

Holmstedt (1980) and May (1973) reviewed reports of three other industrial explosions: a BASF factory in Ludwigshafen, Germany in 1953; the Coalite and Chemicals plant in England in 1968; and a 2,4,5-T producing factory in Amsterdam in 1963. Severe chloracne was the most common symptom among the exposed workers. Nervous system and unspecified internal organ damage were reported in the German workers. Clinical examinations were performed on 14 of the ~90 workers in the Coalite and Chemicals plant at the time of the explosion. Eleven of the 14 had altered liver function, altered hematological parameters or glucosuria.

An accident at the ICMESA plant at Seveso, Italy, in 1976, resulted in the exposure of at least 8655 workers and nearby residents when a reactor vessel used in manufacturing 2,4,5-T exploded (Garattini, 1982). From this population, there were a total of 447 reported cases of chloracne, along with complaints of nausea, vomiting, headache, diarrhea, hyperhidrosis and irritation of the eyes (Taylor, 1979; Gianotti, 1977; Crow, 1981). Serious cases of chloracne occurred in children within several weeks of the exposure.

The Lombardy Regional Authority has compiled extensive data regarding the health effects of 2,3,7,8-TCDD on children and adults at Seveso (Pocchiari et al., 1979; Boeri, 1978; Chiappino et al., 1978; Sirchia, 1978). Reduced peripheral nerve conduction velocities occurred in both adults and children, with a correlation between the incidence and the distance from the plant. Total serum complement activity, lymphocyte blastogenic response and peripheral blood lymphocytes were elevated in children exposed to the accident (Tognoni and Bonaccorsi, 1982). The limited number of studies regarding the immunological effects of 2,3,7,8-TCDD in adults have not revealed any reduction in immunocapability (Reggiani, 1980; May, 1982).

Caramaschi et al. (1981) reported an increase in the frequency of headaches, eye irritation, gastrointestinal tract symptoms and abnormal γ-GT, serum GPT and aminolevulinic acid levels in children, living in the Seveso area, who developed chloracne. Increased urinary glucaric acid levels, indicative of increased microsomal enzyme activity, were found in children 3 years after the accident (Ideo et al., 1982).

Six children dermally exposed to contaminated soil (30 ppm, 30 mg/kg soil) in horse arenas in Eastern Missouri developed headaches, skin lesions and polyarthralgia (Kimbrough et al., 1977). In the most severe case, epistaxis and lethargy were reported.

Numbness of the extremities, skin rashes and irritation, liver dysfunction, weakness, loss of sex drive and psychological changes have been associated with exposure to 2,3,7,8-TCDD and other dioxins, which occur as contaminants in Agent Orange, in veterans and residents of Vietnam. The relationship between exposure to 2,3,7,8-TCDD and the development of these symptoms is, as yet, unknown (Holden, 1979; Bogen, 1979).

Stevens (1981) estimated the cumulative minimum toxic dose of 2,3,7,8-TCDD in man to be 0.1 μg/kg, based on analogy to 2,3,7,8-tetrachlorodibenzo-p-furan. Given the conditions prevailing in Vietnam, Stevens estimated that 5 years of exposure to Agent Orange would be required to reach this dose level.

Cytogenetic studies of lymphocytes from individuals exposed to 2,3,7,8-TCDD, usually by exposure to contaminated 2,4,5-T, have been performed. In two of the studies involving exposure during the Seveso incident, no increase in the incidence of chromosomal aberrations was observed as compared with nonexposed controls (Reggiani, 1980; Mottura et al., 1981). In a third such study, changes suggestive of a mutation in functional nucleolar organizing regions of lymphocyte chromosomes were observed (DiLernia et al., 1982), but this assay has yet to be validated. Workers exposed to the herbicides 2,4,5-trichlorophenoxy-ethanol (2,4,5-TCPE) and Buminol, contaminated with 2,3,7,8-TCDD, were found to have an increased incidence ($p<0.001$) of chromatid-type and unstable chromosomal aberrations in peripheral lymphocytes (Czeizel and Kiraly, 1976). No increased incidences of chromosomal aberrations were observed in soldiers exposed 10 years previously to Agent Orange, as compared with unexposed subjects (Mulcahy, 1980). With the exception of the Mulcahy (1980) study, the studies described in this paragraph were performed within a few weeks of exposure. All studies involved exposure to other chemicals and exposure levels were not characterized.

Thirty-five cases of various types of cancer were reported in 570 Vietnam veterans. The cancers (including 1 lung, 3 kidney and 2-3 in testes) were attributed to 2,3,7,8-TCDD exposure during "Agent Orange" (a mixture of 2,4-D, 2,4,5-T and 30-50 ppm 2,3,7,8-TCDD) sprayings in Vietnam (Holden, 1979). These data are of little value because the 570 veterans were not selected at random; instead, they were selected because they complained of symptoms related to Agent Orange exposure. According to the study, there was no reference group with which to compare these statistics.

A few occurrences of soft tissue sarcoma have been reported among chemical industry workers in the United States who were exposed to varying levels of 2,4,5-T, 2,4,5-TP, chlorophenols and 2,3,7,8-TCDD contaminants (Cook et al., 1980; Moses and Selikoff, 1981). Honchar and Halperin (1981) reported that 3 of 105 deaths among phenoxy acid workers reported by two chemical companies were from soft tissue sarcoma. This represents a 2.9% mortality rate due to this cancer while only 0.07% of the deaths among males in the United States were due to soft tissue sarcoma. Cook (1981) reported a fourth case of malignant fibrous histiocytoma in a phenoxy acid chemical worker. For a similar industrial setting, except that 2,3,7,8-TCDD levels were <1 ppm, Ott et al. (1980) reported no increase in the cases of cancer among 204 chemical workers. This study reported on 22 deaths among the workers, however, and >75% of the men worked for <12 months in a job involving some 2,3,7,8-TCDD exposure.

Epidemiological Studies

Poland et al. (1971) reported the results of a health survey conducted among 73 workers who had been exposed to 2,4,5-T, di- and trichlorophenols, dioxin contaminants and 2,4-D. Thirteen of the workers developed moderate to severe cases of chloracne. Other complaints included minimal active acne, eye irritation, hyperpigmentation, hirsutism and gastrointestinal symptoms. These effects could not be attributed to exposure to any specific compound. In a study by Walker and Martin (1979), chloracne and elevated γ-GT, triglyceride and cholesterol levels have been associated with occupational exposure to 2,3,7,8-TCDD. Other studies have reported a correlation between the extent of occupational exposure to 2,3,7,8-TCDD and the development of chloracne (Ott et al., 1980; Cook et al., 1980). Neuropathies have been reported in workers involved in the production of 2,4,5-sodium trichlorophenoxyacetate and trichlorophenoxyacetate butylester (Pazderova-Vejlupkova et al., 1981) and phenoxy acid herbicide (Singer et al., 1982). Dioxin contaminants were suspected to be the causative agents in these cases.

A positive association between 2,4,5-T exposures and increases in birth defects or abortions has been reported in human populations in Oregon (U.S. EPA, 1979a), New Zealand (Hanify et al., 1981) and Australia (Field and Kerr, 1979). A lack of any such association has been reported in human populations in Arkansas (Nelson et al., 1979), Hungary (Thomas, 1980), New Zealand (Dept. of Health, New Zealand, 1980; McQueen et al., 1977) and Australia (Aldred, 1978). Almost all of the reports are geographic correlation studies, and because of the uncertainties inherent in this type of epidemiologic investigation, as well as the difficulties in distinguishing the effects of 2,4,5-T from those of 2,3,7,8-TCDD contamination, none of the reportedly positive associations unequivocally identify either 2,4,5-T or 2,3,7,8-TCDD as the causative agent. Similarly, the reportedly negative associations do not rule out 2,4,5-T or 2,3,7,8-TCDD as potential teratogens or abortifacients in humans.

Based on a report of a high incidence of abortions in a small group of women living around Alsea, Oregon, who may have been exposed to the herbicide 2,4,5-T from aerial spraying (Smith, 1979), the U.S. EPA (1979a) initiated a study, often referred to as the "Alsea II study," to determine if spontaneous abortion rates differed between the exposed and unexposed population, if spontaneous abortion rates evidenced seasonal variation in these two groups, and if such seasonal variations were associated with 2,4,5-T spray application.

Spontaneous Abortion Rate Index, as defined by the U.S. EPA, is "basically the ratio of the number of hospitalized spontaneous abortions to the number of births corresponding to the spontaneous abortions, based on the residence zip code of the women contributing to each event." Upon completion of the study, the U.S. EPA concluded that (1) the 1972-1977 Spontaneous Abortion Rate Index for the study area was significantly higher than in the Rural Control Area or the Urban area; (2) there was a statistically significant seasonal cycle in the abortion index in each of the areas with a period of ~4 months. In particular there was an outstanding peak in the study area in June; and (3) there was a statistically significant correlation between the Spontaneous Abortion Rate Index and spray patterns in the study area when a lag-time of 2 or 3 months was included. The U.S. EPA concluded however, "This analysis is a correlational analysis, and correlation does not necessarily mean causation."

Milby et al. (1980), citing three critiques of the Alsea II study that were not published in the open literature, state that the statistical method and basic design of the Alsea II study were sufficiently flawed to make this study of no use in human risk assessment. The Alsea II study has also been reviewed by a panel of epidemiologists who, in a published report of their meeting, also concluded that the basic design of the study was inadequate to demonstrate either an effect or absence of an effect of exposure to 2,4,5-T (Coulston and Olajos, 1980). The major inadequacies of the study were that the data collection methods were biased and would likely result in the underestimation of abortions, particularly in the urban area (the incidence of abortions in all three groups was within the expected background rate of 8-15%); only a small portion of the area from which the exposed subjects were selected was actually sprayed with 2,4,5-T; and the study was not controlled for other factors such as age, smoking habits and alcohol consumption, which may affect the spontaneous abortion rate. Based on a report by Smith (1979), the U.S. EPA is attempting or has attempted to correlate 2,3,7,8-TCDD levels in the affected areas with the observed rate of abortion. No published reports have been encountered on the outcome of this effort.

In the only other report encountered on a population in the United States, Nelson et al. (1979) noted a general increase in the reported incidence of facial cleft in both high and low exposure groups in Arkansas from 1948 to 1974. In this study, exposure estimates were based on average rice production in different areas of Arkansas, and the incidence of cleft palate was determined by screening birth certificates and checking records of the Crippled Children's Services. No consistent exposure/effect correlations were noted, and the general increase with time in the incidence of facial clefts was attributed to better reporting procedures; however, there does not have to be a direct correspondence of malformations in human beings and experimental animals.

Of the four reports available from New Zealand (Dept. of Health, New Zealand, 1980; McQueen et al., 1977; Hanify et al., 1981; Smith et al., 1982a), the report by the Department of Health is essentially anecdotal, involving two women who gave birth to malformed children (one with an atrial septal defect and a malformation of the tricuspid valve of the heart and the other with biliary atresia). In both cases, exposure to 2,4,5-T could not be ruled out. Based on an analysis of spraying records, the time course of the pregnancies and plant damage near the women's homes, however, the Department of Health, New Zealand (1980) concluded that there was insufficient evidence to implicate 2,4,5-T spraying as a causative factor. Even if the spraying had been implicated, a lack of information on 2,3,7,8-TCDD levels in the spray and the absence of any monitoring data on 2,4,5-T or 2,3,7,8-TCDD would limit the usefulness of this report.

The study by McQueen et al. (1977) is not published in the open literature but is summarized by Milby et al. (1980). According to the summary, McQueen et al. (1977) "...examined the epidemiology of neural-tube defects in three areas in New Zealand and concluded 'there is no evidence to implicate 2,4,5-T as a causal factor in human birth defects.'" No additional details are provided.

Hanify et al. (1981) performed an epidemiologic study in Northland, New Zealand, in areas where spraying of 2,4,5-T was conducted by various companies for a number of years. The rate of birth defects was obtained from an examination of hospital records in seven mutually exclusive areas on a monthly basis over a period extending from 1959-1977. The rate of birth defects from 1959-1965 represented the rate for a nonexposed population since this occurred before the use of 2,4,5-T, while the incidence of birth defects from 1972-1976 represented the rate for the exposed population. During the time of the survey there were 37,751 births, 435 stillbirths, 264 deaths shortly after birth and 510 congenital anomalies. Three categories of birth defects, heart abnormalities, hypospadias and epispadias, and talipes, had elevated rate ratios of >1 (p=0.05) in comparisons between the exposed (1972-1976) and control (1959-1965) populations. Exposure estimates were made for the seven areas and for different years using company records of aerial spraying and a model that factored in assumed fractional removal rates/month (this factor was assumed to be either 1.0 or 0.25). Comparisons of the rate of specific malformations with exposure demonstrated a statistically significant association between the occurrence of talipes and exposure when the fractional removal rate was assumed to be 0.25. There was, however, no statistically significant association where 1.0 was used as the fractional removal rate.

Smith et al. (1982a) investigated the outcome of pregnancy in families of professional 2,4,5-T applicators and agricultural contractors in New Zealand. Agricultural contractors were chosen as the control population since both sprayers and contractors were of the same economic group with similar outdoor occupations. The survey was conducted by mail with 89% of the chemical applicators responding and 83% of the agricultural contractors responding to questions asking whether they used 2,4,5-T and its temporal relationship to reproductive histories regarding birth, miscarriages, stillbirths and congenital defects. The relative risks of congenital defects and miscarriages were 1.19 (0.58-2.45% confidence limits) and 0.89 (0.61-1.30% confidence limits) for the wives of chemical sprayers as compared with the wives of agricultural contractors. These data indicate that exposure of fathers and mothers (e.g., while cleaning clothes) had no effect on the outcome of pregnancy. Biases that may have affected the results, such as the age of the mother at childbirth, smoking habits and birth to Maori parents were investigated and eliminated as possible confounders.

The two reports from Australia (Aldred, 1978; Field and Kerr, 1979) also present apparently conflicting results. The report by Aldred (1978) is not published in the open literature, but the following summary is taken from

Milby et al. (1980): "The report concluded that birth defects in a group of babies born in the [Yarram] district in 1974 and 1976 could not be attributed to exposure to 2,4,5-T or 2,4-D." Additional details that might be useful in assessing the rationale for this statement are not provided in the summary. The report by Field and Kerr (1979) plotted the incidence of neural-tube defects (anencephaly and meningomyelocele) in New South Wales, Australia, over the years 1965-1975, and the previous years usage of 2,4,5-T in all of Australia. The authors noted a decrease in the incidence of neural-tube defects expected on the basis of the plotted line in 1975 and 1976, when Australia instituted monitoring of 2,4,5-T to ensure a 2,3,7,8-TCDD level <0.1 ppm. The data were not tested for significance, although Field and Kerr (1979) indicate that they consider the epidemiological data on neural-tube defects to be "relatively complete"; however, they do not comment on the increasing incidence of neural-tube defects with time and whether or not an increase in the thoroughness of reporting neural-tube defects could have contributed to the apparent correlation of 2,4,5-T exposure with these defects. A visual replotting of the data suggests that the incidence of cleft palate correlates better with 2,4,5-T usage than with time. Nonetheless, the appropriateness of correlating 2,4,5-T usage in all of Australia with the incidence of defects in one area of Australia is questionable.

Thomas (1980) used an approach similar to that of Field and Kerr (1979) on data from Hungary. One major difference, however, is that Thomas (1980) compared the incidence of stillbirths, cleft lip, cleft palate, spina bifida, anencephalus and cystic kidney disease in all of Hungary between 1976 and 1980 with 2,4,5-T use in 1975 in all of Hungary. Because Hungary requires compulsory notification of malformations diagnosed from birth to age 1 year, because a relatively large percentage (55%) of the Hungarian population lives in rural areas where 2,4,5-T exposure may be expected to be greatest, and because annual use of 2,4,5-T in Hungary had risen from 46,000 kg in 1969 to 1,200,000 kg in 1975, Thomas (1980) considered Hungary to be "...probably the best country in which to examine possible health effects of this herbicide." In any event, all indices of birth defect rates decreased or remained stable over the period of study.

In addition to contamination of 2,4,5-T being a potential source of 2,3,7,8-TCDD exposure, 2,3,7,8-TCDD is also an inadvertent contaminant of 2,4,5-trichlorophenol (TCP). Chronic exposure to 2,3,7,8-TCDD may occur during the manufacture of TCP and high level acute exposure to 2,3,7,8-TCDD has occurred after an accident in July 1976, at the ICMESA TCP chemical factory in Seveso, Italy (Bonaccorsi et al., 1978). In this accident, the reaction used to produce TCP became uncontrolled, producing conditions favorable for 2,3,7,8-TCDD formation before venting the contents of the chemical reactor into the atmosphere. The resulting cloud of chemicals settled over a heavily populated area. Although the amount of 2,3,7,8-TCDD released was not known, the reported cases of chloracne, a symptom of acute exposure to 2,3,7,8-TCDD, indicated that exposure to 2,3,7,8-TCDD had occurred. Some preliminary results are available from epidemiologic studies of reproductive events in the inhabitants of Seveso, and recently a study has become available on the reproductive history of men employed in the chemical manufacturing industry with possible chronic exposure to 2,3,7,8-TCDD (Townsend et al., 1982).

Epidemiologic studies to determine the reproductive effects in individuals exposed to 2,3,7,8-TCDD and TCP following the accidental contamination of a populated area around Seveso, Italy, are not completed. The incidence of spontaneous abortions occurring between March 1976 and January 1978 have been reported for inhabitants in the area around Seveso by Bonaccorsi et al. (1978), Reggiani (1980) and Bisanti et al. (1980). The spontaneous abortion rate in the contaminated area for the three trimesters following the accident was 13.1, 11.0 and 13.05%, which was similar to the worldwide 15-20% frequency of spontaneous abortion. Subdividing the contaminated area into highly, moderately, and least contaminated, and examining the rates for each area individually, also failed to demonstrate any change in the spontaneous abortion rate. The incidence rates of malformations were also examined; however, the numbers were too small for meaningful assessment. There are several reasons why these studies would not indicate that the effect of 2,3,7,8-TCDD exposure in this accident had no effect on human reproduction. The authors note that there are many difficulties in interpreting these data. The incidence rates of spontaneous abortions and birth defects were not adequately available for the region before the accident as a result of suspected under-reporting. The inadequate reporting even after the accident was due to political turmoil with regard to the management of health services. Also, an unknown number of pregnancies were surgically aborted for fear of 2,3,7,8-TCDD-induced birth defects. In a recent review of the progress of epidemiologic investigations of the Seveso accident, Tognoni and Bonaccorsi (1982) indicated that the data on spontaneous abortions and malformation rates still needed verification and that these data were too preliminary to allow for conclusions.

Townsend et al. (1982) investigated the reproductive history of wives of employees potentially exposed to 2,3,7,8-TCDD during chlorophenol production. A total of 930 potentially exposed males were identified who had worked for ≥ 1 month between January 1939 and December 1975 in a job with potential 2,3,7,8-TCDD exposure. Exposure estimates of low, moderate and high were made by an industrial hygienist primarily from job description and surface contamination data; however, the high potential exposure group was reserved for process workers during 1963-1964 when changes in operations resulted in a number of cases of chloracne. The control population was an equal number of male employees not involved in any process that might cause exposure to 2,3,7,8-TCDD and matched for date of hire. In these groups, 586 wives were identified and 370 agreed to participate as the exposed group, while 345 wives in the control group agreed to participate. After identification of the participants, a personal interview was conducted with the wives to determine pregnancy outcome. Of the total of 737 conceptions in the exposed category and 1785 conceptions in the control category (conception that occurred in the exposed group before availability of work records indicating potential exposure to 2,3,7,8-TCDD were placed in the control group), there was no statistically significant increase in spontaneous abortions, stillbirths, infant deaths or selected congenital malformations. Sample sizes were too small to provide meaningful data if the populations were subdivided by extent of exposure. It was suggested that many confounding factors could account for these negative results, such as the inappropriate selection of the populations, unidentified covariables and insufficient power; however, the authors maintained that these results were consistent with animal data, which report that paternal exposure to 2,3,7,8-TCDD does not affect the conceptus.

Poole (1983), in testimony before the House Committee on Science and Technology, described a reanalysis of the primary data used by Townsend et al. (1982). In this reanalysis, the rate of cleft palate and cleft lip were reported to be elevated by 1.9 (90% confidence intervals of 1.0-3.6) in the years 1971-1974 for both the control and exposed groups (the comparison population was not described). At the same House Committee hearing, Houk (1983) presented data from the Birth Defect Monitoring Program of the Center for Disease Control on the yearly rate of cleft palate alone or cleft lip with or without cleft palate for births in Midland County, Michigan (the site of a chlorophenol production facility) during the years 1970-1981. The data indicated an increased rate for these defects of between 50 and 100% in the years 1971-1975, with the rate returning to expected from 1976-1981. The observed increase was only statistically significant if the rates for cleft palate alone and cleft lip with or without cleft palate were combined; however, it was the opinion of Houk (1983) that these defects should not be combined since the causal mechanism may be different. The Michigan Department of Public Health (1983a) also reported these results and, in addition, demonstrated that the same results occurred if the comparison was made with other counties in Michigan as well as with the general population of the United States. It was noted in this report that "runs" of increases in oral cleft for successive years have occurred in six other counties with no obvious potential for chemical exposure described. The Michigan Department of Public Health (1983a) interpreted the data to indicate that a more detailed case control study was necessary to determine if any common factors may exist, such as exposure to chemicals contaminated with 2,3,7,8-TCDD.

Several investigators have suggested that 2,3,7,8-TCDD is the causative agent of excess liver carcinoma and soft-tissue sarcoma associated with occupational or accidental exposure to phenoxyacetic acid herbicides. Direct evidence is limited since the dioxins usually occur in conjunction with other chemicals and quantitative exposure data are not available.

Observations of an unusual occurrence of relatively rare soft-tissue sarcomas were first made by Hardell (1977). Of some 87 patients seen from 1970-1976 at the Department of Oncology, University Hospital, Umea, Sweden, seven individuals with soft-tissue sarcomas were identified. All seven had had occupational exposure to phenoxy acids 10-20 years earlier. The tumors were 2 leiomyosarcomas, 1 liposarcoma, 1 rhabdomyosarcoma, 1 myxofibrosarcoma and 2 additional sarcomas of which the histopathology was uncertain, but one was probably a neurofibrosarcoma and the other a rhabdomyosarcoma. The clustering of this rare tumor type among these patients prompted the author to suggest that epidemiological studies be done to determine if exposure to phenoxy acids and the impurities they contain are related to the occurrence of soft-tissue sarcomas.

Zack and Suskind (1980) reported a soft-tissue sarcoma death in a cohort study of workers exposed to 2,3,7,8-TCDD in a trichlorophenol process accident in Nitro, West Virginia. This tumor, a fibrous histiocytoma, was noted by the author as a rare event. This study, referred to as the Nitro study, is discussed later.

Cook et al. (1980) in a cohort mortality study of 61 male employees of a trichlorophenol manufacturing area, who exhibited chloracne following a 1964 exposure incident, noted four deaths by the end of his study period, one of which was due to a fibrosarcoma. The authors did not seem to attribute any special significance to this finding at the time.

Ott et al. (1980) in a cohort mortality study of 204 employees exposed to 2,4,5-T during its manufacture from 1950 to 1971, found no soft-tissue sarcomas among 11 deaths that had occurred by 1976. One 1 of these 11 deaths was due to a malignant neoplasm.

In a review of the studies of Zack and Suskind (1980), Cook et al. (1980), an unpublished study by Zack (in which a liposarcoma was found), and a study by Ott et al. (1980), Honchar and Halperin (1981) noted 3 (2.9%) soft-tissue sarcomas in a total of 105 deaths, compared roughly to 0.07% deaths due to soft tissue sarcoma expected in United States males 20-84 years old, (ICD 171, 8th Revision, 1975)* indicating an unusual excess of such tumors. The researchers underestimated the results because of the possiblity that some soft-tissue sarcomas may have been coded to categories other than ICD 171. Individually, none of the reported case studies reported a significant excess of soft-tissue sarcomas. Cook (1981) found an additional malignant fibrous histiocytoma after a later review of the medical records from his earlier cohort study. Cook, who was familiar with the three earlier cases, noted that frank chloracne occurred previously in two cases of the four having a diagnosis of malignant fibrous histiocytoma. A third person diagnosed as having a fibrosarcoma worked in a trichlorophenol

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*Department of Health, Education, and Welfare. U.S. Public Health Service. National Center for Health Statistics of the United States, 1974. Vol. II. Mortality, Part A.

(TCP) process area contaminated with 2,3,7,8-TCDD. This individual exhibited facial dermatitis but no diagnosis of chloracne. The fourth case (diagnosed as a liposarcoma) was an individual who had been employed earlier in a plant producing 2,4,5-T. Cook (1981) noted that although chloracne was not reported, it could not be discounted. He also noted that all four were smokers and suggested that cigarette smokers with chloracne caused by 2,3,7,8-TCDD exposure may be subject to an increased risk of fibrous soft-tissue sarcomas.

Hardell and Eriksson (1981) discounted this hypothesis by citing that only one of Hardell's seven cases exhibited chloracne before the appearance of the soft-tissue saromcas, and that in their subsequent case control study, they found no difference in smoking habits between his cases and controls.

Moses and Selikoff (1981) reported a fifth soft-tissue sarcoma in a worker employed at the Monsanto Chemical Company at a time when trichlorophenol and 2,4,5-T were being produced. He died of a retroperitoneal neurogenic sarcoma (malignant schwanoma) in 1980 at the age of 58. The employee, prior to his death, in a detailed occupational history said that he believed he was exposed to these chemicals while he was a truck driver, hauler and maintenance worker, but that he did not work in the production of either chemical. He was a nonsmoker and did not have a history of chloracne.

Johnson et al. (1981) treated a father and son with soft-tissue sarcomas (the 33-year-old son was diagnosed as having a fibrosarcomatous mesothelioma, while the 53-year-old father had a liposarcoma). Both were exposed to halogenated phenol derivatives. The author noted that 2,4-dichlorophenol can be a precursor of 2,4-D and 2,4,5-T. The father had had prolonged exposure before the diagnosis. The son supposedly had a shorter latency, according to the author. In neither case was the follow-up time given.

Sarma and Jacops (1981) reported three cases of thoracic soft-tissue sarcoma in individuals who were presumably exposed to Agent Orange while serving in Vietnam. The diagnoses were fibrous histiocytoma, mediastinal fibrosarcoma, and a pleural/diaphragmatic leiomyosarcoma. All three served in areas where defoliants were used at the time. One was drenched with the material in a single spraying.

Bishop and Jones (1981) found two cases of non-Hodgkin's lymphomas of the scalp in a related clinical study of 158 employees of a pentachlorophenol manufacturing plant in Wales. Homologues of 2,3,7,8-TCDD occurred as contaminants at up to 300 ppm at intermediate manufacturing stages and 5 ppm in the final products. Mild, moderate and severe cases of chloracne were seen in many employees, including the two men who subsequently developed lymphomas. Both men worked in processes where exposure to other chemicals occurred, including exposure to aromatic hydrocarbons. The authors reported that only 0.28 tumors of this type could be expected to occur in a group of 158 workers (ICD 200 and 202), although the basis for the computation of expected numbers is not stated.

Olsson and Brandt (1981) noted that of 123 male patients seen at their clinic in Sweden with a recent diagnosis of non-Hodgkin's lymphoma (NHL), 5 had cutaneous lesions as the only clinically detectable manifestation of NHL. Four of them were reported to have had repeatedly sprayed large areas with phenoxy acid herbicides. In the remaining 118 NHL patients, only seven had a similar occupational exposure to phenoxy acids. The authors reported this to be significant at $p<0.001$. Olsson and Brandt (1981) suggested that a relationship exists between cutaneous presentation of NHL and occupational exposure to phenoxy acids, and believed their observations were similar to those of Bishop and Jones (1981).

The total number of workers with these illnesses who were exposed to phenoxy acids or chlorophenols or both is small, but considering the rarity of this cancer, it is unusual that so many cases of soft-tissue sarcomas have occurred. A Lancet editorial (Anonymous, 1982) calls this phenomenon "disturbing."

Soft-tissue sarcomas (STS) constitute a collection of heterologous lesions that include both malignant and nonmalignant tumors. Not all of them have their origin in primordial mesenchymal cells. Some exceptions are tumors of peripheral nerves, and neuroectodermal tumors that are classified as STS, but are derived from *non*mesenchymal cells. Classification, grading and staging of STSs is difficult because of the capacity of such cells to differentiate into many different tissues. Fairly precise histogenetic classification of such tumors is accomplished through consideration of growth patterns and cell morphology, and evaluation of intracellular and extracellular products of tumor cells. There are a dozen distinctly different classes of mesenchymal cells that develop into the following six well-defined tissue complexes: fibrous tissue, tendosynovial tissue, adipose tissue, muscle, vessels and bone. STSs can be induced in any of these tissue types (Hajdu, 1983). The classification of STSs for cause of death coding in the ninth and latest revision of the International Classification of Diseases (ICD, 1975) places STSs into one of several categories. But chiefly, they fall into "malignant neoplasms of connective and other soft-tissue" (ICD 171). Lymphosarcomas, retroperitoneal sarcomas and extra skeletal STSs of the bone are coded elsewhere. In some instances, if site is mentioned, it is coded to the site, e.g., leiomyosarcoma of the stomach (ICD 151.9), neurofibroma of the chest wall (215.4).

Questions have been raised concerning the appropriateness of lumping together malignant tumors of different sites and tumor types in order to derive risk estimates. It may not be scientifically appropriate to do so because an elevated risk cannot readily be ascribed to a particular site or type as is usual with most carcinogenic chemicals and substances. Unfortunately, with respect to STSs, tallies of deaths caused by STSs of particular sites and types are not maintained separately by the vital statistics offices because of their rarity, and therefore, it is impossible to derive risk estimates for particular types at given sites. Altogether, ~2000 deaths/year can be attributed to STSs in the United States, most of which are coded to ICD category 171 for purposes of developing incidence and mortality rates for this composite cause. Within ICD 171, individual types that may be correlated with exposure cannot be identified.

A separate problem that potentially could arise from assigning STSs to multiple ICD codes is that incidence and death rates from STSs may be underestimated. Furthermore, risk estimates derived from dividing observed cases (or deaths) by expected cases (or deaths) could be biased upward. This could happen when observed STSs classified to ICD codes other than ICD

171 are lumped together while expected STSs are based upon ICD 171 only. Thus, action of this sort, especially with respect to cohort studies of individuals exposed to dioxin-containing herbicides or chlorophenols or both, could lead to risk estimates that may be biased upward by the inclusion of STSs in the observed category for risk estimation that should be coded to categories other than 171.

Prompted by clinical observations over a 7-year period of malignant sarcomas in seven men with previous occupational exposure to phenoxyacetic acid herbicides (Hardell, 1977), researchers at the Department of Oncology. University Hospital, Umea, Sweden, initiated case-control epidemiologic studies (Cole, 1979) to test the hypothesis of an etiologic association (Hardell and Sandstrom, 1979). Cases were defined as male patients with sarcomas of soft connective tissue, such as smooth muscle (leiomyosarcoma) and fat (liposarcoma). The distribution of tumor types in the two studies is shown in Table VI-1. Sarcomas of tissues, such as bone and cartilage, were excluded as cases. According to the authors, these tumors may have a different etiology and there occurred a different age-distribution in patients with these tumors as compared with that of STS (Hardell, 1983).

Two case-control studies were conducted, the first in northern Sweden (referred to as Study A), and the second in the southern part of the country (Study B). The exposures to the substances of primary interest are shown in Table VI-2. In the north (Study A), occupational exposure to phenoxyacetic acids took place in both forestry and agricultural work. In the south (Study B), these exposures were predominantly agricultural. The phenoxy-

TABLE VI-1

Distribution of Tumor Types in Two Case-Controls Studies of Soft-Tissue Sarcoma

Diagnosis	Tissue of Origin	Percent of Cases Study A[a] (n=52)	Study B[b] (n=110)
Leiomyosarcoma	Smooth muscle	30	23
Fibrous histiocytoma	Subcutaneous connective tissue	17	25
Liposarcoma	Fat tissue	14	6
Neurogenic sarcoma	Nerve tissue	10	4
Angiosarcoma	Blood vessels	8	2
Myxosarcoma	Primitive connective tissue	6	8
Fibrosarcoma	Fibrous tissue	4	8
Other sarcomas		11	24
Total		100	100

[a] Unpublished information supplied by Hardell to EPA (Hardell and Sandstrom, 1979)

[b] Eriksson et al., 1979, 1981

TABLE VI-2

Exposure Frequencies in Two Case-Control Studies of Soft-Tissue Sarcoma

Substance(s)	Percent Exposed			
	Study A		Study B	
	Cases (n=52)	Controls (n=206)	Cases (n=110)	Controls (n=219)
Phenoxyacetic acids only	23.1	6.3	12.7	2.3
Chlorophenols only	11.5	2.4	10.0	3.6
Both	1.9	0.5	0	0
Total	36.5	9.2	22.7	5.9

*Sources: Study A, Hardell and Sandstrom, 1979; Study B, Eriksson et al., 1979, 1981 acetic acids to which exposure occurred consisted predominantly of 2,4,5-T and 2,4-D in both studies. Exposure to 2,4,5-T in the absence of 2,4-D was rarely reported in either study. Exposure to chlorophenols, which contain chlorinated dibenzodioxin impurities (Levin et al., 1976) occurred mostly in sawmill work and paper pulp production. Very few persons reported exposure both to phenoxyacetic acid and chlorophenols in these studies. Of the two predominant phenoxyacetic acids, only 2,4,5-T is known to be contaminated with 2,3,7,8-TCDD. In Study B, a relative risk of 4.9 (90% confidence intervals 1.6-11.1) was found in relation to exposure to phenoxyacetic acid herbicide other than 2,4,5-T (2,4-D, MCPA, mecoprop, dichloroprop).

Relative risks in relation to the three major categories of exposure are shown in Table VI-3.* Studies A and B indicate a risk of developing STSs among workers exposed to phenoxyacetic acids only, chlorophenols only, or phenoxyacetic acids and/or chlorophenols several times higher than among persons not exposed to these chemicals. In each comparison, the relative risk is high ($p<0.005$) and unlikely to have resulted by chance alone.

Since little is known of the etiology of STSs, the consideration of confounding in these studies was largely a hypothetical matter. The authors prevented the effects of age, sex, and place of residence as possible

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*In the analyses considering phenoxyacetic acids only and chlorophenols only, persons exposed to the other categories of substances were excluded. In Study A, the three persons exposed to both chlorophenols and phenoxyacetic acids were included in all comparisons.

TABLE VI-3

Relative Risks of Soft-Tissue Sarcoma in Relation to Exposure to Phenoxyacetic Acids and Chlorophenols in Two Case-Control Studies[a]

	Phenoxyacetic Acids Only		Chlorophenols Only		Phenoxyacetic Acids and/or Chlorophenols	
	Study A	Study B	Study A	Study B	Study A	Study B
Relative risk[b]	5.3	6.8	6.6	3.3	5.7	4.7
90% Confidence interval[c]	2.7-10.2	3.1-14.9	2.8-15.6	1.6-7.0	3.2-10.2	2.7-8.3
Significance level[d]	<0.001	<0.001	<0.001	<0.005	<0.001	<0.001

[a] Source: Study A, Hardell and Sandstrom, 1979; Study B, Eriksson et al., 1979, 1981

[b] Unmatched odds ratio

[c] Test-based method of Miettinen, 1976

[d] Chi square statistic, no continuity correction, one-tailed test

confounding factors in the selection of controls.* Because of the high correlation between exposure to the substances of interest and employment in agriculture and forestry, a possible alternative hypothesis could be developed that some other unknown factor present in these occupations was responsible for the elevated relative risks.

To test this hypothesis, it is possible to calculate the relative risk in relation to the phenoxyacetic acid exposure in Study B, restricting the analysis to workers within agriculture and forestry. The result is a relative risk of 6.1 (90% confidence interval 2.4-15.4). This finding strongly suggests that some confounding risk factor for STS distributed throughout agriculture and forestry work was not responsible for the overall increase in risk found in relation to phenoxyacetic acid exposure.

Because exposure histories were obtained by means of questionnaires and interviews, the major potential source of bias in these studies stems from the need to rely upon the personal recollection of cases and controls for exposure histories. The published papers indicate that the researchers paid a great deal of attention to this potential problem and specific efforts were made to avoid it during the conduct of the study.

In addition, the relative risk calculated by considering the agriculture and forestry workers who did not report exposure to phenoxyacetic acids or chlorophenols and comparing them with unexposed persons in other occupations was 0.9 (90% confidence interval 0.3-2.4) in Study B. This suggests that little recall bias was present (Axelson, 1980).

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*Controls were matched individually to cases on the basis of these factors. Unmatched analyses are presented in Table VI-3 for the sake of simplicity. The matched-method relative risks for exposure to phenoxyacetic acids and/or chlorophenols were 6.2 ($p<0.001$) in Study A and 5.1 ($p<0.001$) in Study B.

In an update of their earlier study, Eriksson et al. (1981) obtained information on the effects of phenoxy acids in the absence of the impurities--polychlorinated dibenzodioxins and dibenzofurans. The risk ratio given exposure to phenoxy acids free of polychlorinated dibenzodioxins and dibenzofurans equaled 4.2 based upon 7 out of 14 respondents who indicated exposure to phenoxy acid herbicides. When consideration was given to persons exposed to only phenoxy acids that contain such impurities, the relative risk was 17.0. A description of the basis for the determination of exposure or nonexposure to dioxins is not well presented in this study.

The authors concluded that exposure to phenoxy acids and chlorophenols "might constitute a risk factor in the development of soft-tissue sarcomas." This risk relates not only to 2,4,5-trichlorophenoxy acids containing dioxin impurities, but to other phenoxy acids as well. Some doubt was raised concerning the possible misclassifications of individuals who were exposed to phenoxy acids free of polychlorinated dibenzodioxins [i.e., in particular, "dichoroprop" in the Eriksson et al. (1981) study]. In a recent communication from Hardell (1983), Eriksson recalculated risk estimates after reclassifying dichoroprop-exposed cases and controls into the category of probable exposure to phenoxy acids contaminated with polychlorinated dibenzodioxins and removing them from the nonexposed category. The new estimates were 4.0 based upon 5 out of 8 respondents who were exposed to phenoxy acids free of contamination and 10.9 for those exposed to contaminated phenoxy acid. The first estimate was of only borderline significance utilizing the Mietinen test based statistic, thus, weakening any finding that the risk of STS extends to phenoxy acids free of dioxin.

In a cohort mortality study Cook et al. (1980), studied 61 males involved in a 1964 exposure incident who had chloracne caused by absorption of 2,3,7,8-TCDD. The skin lesions characterizing chloracne ranged from a few comedones on the back of one employee (predating his entry into the process area where exposure could occur) to severe cysts and comedones over the faces, scalps, ears, necks and backs of the remaining employees of the group. Since the main route of exposure was not through the respiratory tract, no measurements of dioxin in the air were provided by the author. On the other hand, the author did divide the cohort of 61 males into potentially "high" vs. "low" exposure by place of work based upon dermal exposure, although not stated. Vital status was traced from the data of the incident through 1978. Altogether only 4 deaths were observed by the end of the follow-up vs. 7.8 expected. Of these, 3 were cancer deaths vs. 1.6 expected. The remaining death was hypersensitive heart disease vs. 3.8 expected. The histopathologic causes of death of the three cancer victims were 1) fibrosarcoma, 2) glioma with metastases, and 3) adenocarcinoma. The authors report that all three victims smoked a minimum of one pack of cigarettes a day for "many years." Not enough information is provided by the authors to conclude that any of these four deaths were smoking related. Site of tumor is not mentioned in the cancer deaths.

Cancer mortality was slightly elevated in this cohort. This study had low sensitivity and lacked a sufficient latent period. This increased mortality was not attributable to any particular cause and no deaths were attributable to liver cancer. Additionally, the authors state that only one of the cancer deaths possessed "documented" evidence of chloracne, although this appears to be at variance with the definition of the cohort, which was reported by the authors to consist of males who reported to the medical department with skin conditions subsequently "diagnosed as chloracne." The authors concluded that the latency period was sufficient to "allow the identification of a potent human carcinogen," since it "exceeded 14 years." Orris (1981) noted that in the Hardell and Sandstrom (1979) study the authors stated that the latent period for soft-tissue tumors may be as long as 27 years and for many, over 14 years. In any case, Hueper and Conway (1964) noted that the latent period for the chemical induction of solid malignant tumors in man exceeds 15 years and is probably <30 years.

Smith et al. (1982b) conducted an initial case-control study of 102 males identified from the New Zealand Cancer Registry as having STSs (ICD 171) between 1976 and 1980. For each case, three controls each with another form of cancer were matched by age and year of registration. The selection of cancer controls from the same registry was done to eliminate recall bias or interviewer bias or both. The distribution of histological types in the cases is given in Table VI-4. An interview to elicit occupational history information was accomplished by telephone either to the next of kin or the patient himself, if he was well enough, although the information was not used in this preliminary analysis.

Comparisons between cases and controls were accomplished by use of occupational groupings according to the Standard Classification System of

TABLE VI-4

Distribution of Histological Types of Soft-Tissue Sarcomas*

Cell Type	Number of Cases	Percent
Fibrosarcoma	25	24
Liposarcoma	20	20
Rhabdomyosarcoma	9	9
Leiomyosarcoma	7	7
Malignant Histiocytoma	6	6
Other	22	21
Unspecified	13	13
Total	102	100

*Source: Smith et al., 1982b

New Zealand focusing on those occupational groups with a potential for exposure to phenoxy herbicides and chlorophenols. Expected cases for each major occupational classification were derived based upon the occupational distribution of the controls. The authors found no unusual excess of cases of STS in any major occupational category. In agriculture, forestry and fishing, 14 cases were observed vs. 14.0 expected. In laborers, production and transport workers, 35 cases were observed vs. 37.0 expected. A further breakdown of these two broad categories into finer subcategories within the major occupational categories revealed no significant excesses. The study, however, is not useful in assessing the risk of STS from exposure to phenoxy acids or chlorophenols for several reasons. First, as was pointed out by the authors but subsequently dismissed by them as having not much of an influence, is the possibility that movement from one major occupational category to another over the time period involved for latent conditions to manifest themselves could introduce a negative bias into any estimates of relative risks. The latency for STS was suggested to be a minimum of 15 years (Hueper and Conway, 1964).

The finding of no switching from one occupational category to another that was noted in the "first 20 interviews" in which a change could be noted is not necessarily indicative of fidelity to the same job over long periods in all 408 cases and controls. Information identifying a change may be lacking in those cases and controls if in fact one did occur possibly for several reasons, (separation of the earlier work history from the latter; purging of earlier employment records, etc). Besides, the "first 20 interviews" where a change could be noted is not necessarily representative of the entire cohort in any case.

Furthermore, the authors do not know absolutely that any of their cases and controls were exposed to phenoxy acids or chlorophenols, since apparently no effort was made to confirm "potential" exposures. Only differences in occupational classification were noted where "potential" cases or controls could have had exposure to the dioxin-containing herbicides. It was pointed out that the risk estimates noted do not "preclude" the possibility that an association may be found in this study when the cases and controls (or surviving kin) are interviewed for chemical spraying at a later time. The authors themselves conclude that the preliminary study results "should not be taken as substantial evidence against the hypothesis that phenoxy herbicides and chlorophenols may cause human cancer" (Smith et al., 1982b).

The distribution of tumor types differed considerably from the Hardell and Eriksson et al. (1981) study to the Smith et al. (1982b) study. Leiomyosarcomas, malignant histocytomas, neurogenic sarcomas and myxosarcoma seem to predominate in the Hardell and Eriksson (1981) study, whereas fibrosarcomas and liposarcomas appear prominently in the Smith et al. (1982b) study. More attention should be devoted to the study of the distributions of STS types in registry data everywhere in order to determine if such variations in the reporting of STS types are random occurrences. It is possible that the cancer effect of exposure to phenoxy herbicides may be narrowed to just certain types of STSs, the predominant ones in the Swedish studies.

Smith et al (1983) conducted another case-control study of STSs in males that were reported to the New Zealand Cancer Registry by Public Hospitals between 1976 and 1980. The author matched one cancer control randomly chosen from the registry with each case, initially starting with 112 of each. Controls were matched for year of registration and by date of birth ±2 years. Inquiries were made by the authors with the hospital consultant, family doctor, and finally the next-of-kin or patient if alive. Telephone interviews were conducted by only one interviewer who had no knowledge of the patients cancer history and were completed on 80 cases and 92 controls. Because some 32 potential cases (14 ineligible) and 20 controls were excluded or lost from the study for various reasons, it raises a question whether control of confounding by age and year of registration was maintained in the final group of 172 cases and control included in the analysis. Presumably the corresponding "matched" case or control to each of the 52 lost members of the total study group were not excluded. However, since the span of registration was only 5 years, not much age confounding could occur.

Patients were classified as having had potential exposure to phenoxyacetic acids if they had definite, probable or possible exposure to phenoxyacetic acid through spraying or hand contact. The actual chemical was identified only in some instances. The authors concluded in all remaining situations that if the member sprayed "gorse" or "blackberries," this was tantamount to potential exposure to phenoxyacetic acid. Smith et al. (1983) calculated elevated but nonsignificant relative risks of exposure to phenoxyacetic acid ranging from 1.3 in those individuals who were "probably exposed" for a minimum of 5 days not in the previous 10 years before cancer registration to 1.6 in individuals "probably exposed" for a minimum of 1 day not in the previous 5 years before cancer registration. When risk ratios were calculated after stratifying by year of birth and whether or not the patient or a relative was interviewed, the rates increased to 1.7 (from 1.6) in the latter and 1.4 (from 1.3) in the former calculation, although still nonsignificant. If the numbers would allow, it would be of interest to repeat the above calculations excluding only those with potential exposure occurring only within the 15-year period just before cancer registration. The small numbers that remain following the 15-year lapse precludes such an analysis. Furthermore, the categories of exposure "probably or definitely" exposed for ≥1 day or even 5 days raises a question whether any of the cases or controls could really be said to have ever come in contact with enough phenoxyacetic acid to justify such a designation. It could be that, in fact, potentially exposed individuals in New Zealand have had little or no contact with the herbicide.

The authors did conclude that the finding of a relative risk of 1.7 in individuals with ≥1 day exposure not in the last 5 years cannot be entirely discounted. But then the authors state that if the length of exposure was 5 days or more prior to 10 years before cancer registration they would expect an increase; since they do not see an increase, there is no evidence of a "real causal link." One might ask whether this is a suitable criterion for providing evidence of a causal association. Perhaps a more valid group for study would be one where the potential exposure was considerably longer than "5 days" and >15 years before initial cancer registration. As a subtle justification for the finding of no significant risk in workers exposed in phenoxy acids, the authors (Smith et al., 1983) allude to the fact that there are currently 500 full-time workers registered in New Zealand who do full time ground spraying and altogether some 2000 workers who were at some time professionally involved in phenoxyacetic acid herbicide spraying from the air or ground with exposure "very much greater" than that of patients in this study. This kind of argument has appeal if these workers could be shown to have had their exposure sufficiently far in the past that latency considerations could be adequately addressed. However, the real question again remains how much real exposure did those patients in the study have 10-15 years earlier, and in what numbers. The authors remark that it is surprising that they found no STS victims who had ever worked full-time in phenoxyacetic acid herbicide spraying. Perhaps they have not yet been observed for a long enough period. However, as was pointed out by the authors, the findings do not support the hypothesis that exposure to phenoxyacetic acid herbicides causes STS. But neither do they support a negative finding without better documentation regarding actual exposure and time of actual exposure. The author does not (Smith, 1983), however, state that his documentation of exposure to 2,4,5-T (and 2,4-D) is at least as good as that in the Hardell study, and that although Hardell noted higher relative risks of <30 days exposure, Smith (1983) did not. Hence the paradox. Smith does admit the possibility that 2,3,7,8-TCDD contamination might be lower in New Zealand as opposed to 2,3,7,8-TCDD contamination in the Swedish studies. Although, there is no evidence for it. Smith (1983) still maintains that his study shows that exposure to phenoxyacetic acids may not be associated with STS.

Pazderova-Vejlupkova et al. (1981) studied 80 workers involved in the production of 2,4,5-sodium trichlorophenoxyacetate and butylester of trichlorophenoxyacetic acid who subsequently became ill from exposure to 2,3,7,8-TCDD during the period 1965-1968. Only 55 members of this group were followed for 10 years. The remaining 25 either refused participation or moved leaving no forwarding address. Most patients developed chloracne while 11 developed porphyria cutanea tarda. Chief chemical signs were metabolic disturbances, pathologically elevated lipids with abnormalities in the lipoprotein spectrum, and "pathological" changes in glucose tolerance. Other symptoms noted were biochemical deviations consistent with "a mild liver lesion," light steatosis, periportal fibrosis or activation of Kupffer cells, or nervous system focal damage (peripheral neuron lesion in lower extremeties). Altogether six patients were reported to be deceased during this 10-year period, 2 from bronchogenic carcinoma, 1 from cirrhosis, 1 atherosclerosis precipue cerebi and 2 in auto accidents. No STSs or lymphomas were found. Since there was no comparison population with which to estimate relative risk for cancer, the study must be classified at best as clinical with respect to cancer. The six deaths that occurred during the 10-year observation period in the 55 cannot be construed to be associated with exposure to the 2,4,5-T. Because of the small number of cases and the short follow-up period, nothing can be said concerning the association of exposure with cancer, especially specific types of cancer such as STS or non-Hodgkin's lymphoma.

Riihimaki et al. (1982, 1983) studied a cohort of 1926 herbicide applicators formed in 1972 from personnel records of four Finnish employers (e.g., the Forestry Authority, Highway Authority, State Railways and a state-owned electric power company). Chlorinated phenoxyacids had been used since the 1950s in Finland for spraying. They constituted 2:1 mixtures of emulsified esters of 2,4-D and 2,4,5-T dissolved in water. Analyses from old herbicide formulations dating back to the 1960s revealed that these mixtures contained 0.1-0.9 mg of 2,3,7,8-TCDD/kg formulation).

This cohort of male workers was exposed a minimum of 2 weeks during at least one growing season from 1955-1971. Follow-up continued 9 years through 1980 for mortality but only until 1978 for morbidity. Fifteen individuals could not be traced by 1980. Expected deaths were generated based upon cause- and age-specific national Finnish death rates for 1975. Expected cases were similarly calculated based upon national incidence rates of 1975.

By 1980, 144 deaths had occurred vs. 184.0 expected, a deficit of 22% in observed mortality. Only 26 cancer deaths had occurred vs. 36.5 expected, a 29% deficit. The authors separated out "natural" deaths from the total. The observed residual deaths equaled 39 while the expected deaths equaled 28.7. This excess was of borderline significance. The authors also considered 10-year and 15-year latent periods. Even after 15 years, the deficit of deaths continued to manifest itself both in categories of all causes and total cancers; 35 observed vs. 53.6 expected and 5 observed vs. 11.3 expected, respectively. Similarly, the 7-year follow-up of cancer morbidity revealed 26 cases of cancer vs. 37.2 expected. After 10 years latency, 16 cancer cases were observed vs. 20.1 expected. None of the 26 cancer deaths or 26 cancer cases were of the STS or lymphoma type. (However, only 0.1 STS and 0.5 lymphomas were expected.) In no instance was cancer of any site significantly elevated.

The authors note that this unusual deficit of mortality and morbidity of between 70 and 82% (even after 15 years from initial exposure) is probably a consequence of the "healthy worker effect" in that only able-bodied and healthy individuals were selected into the industry. The fact that the cohort was assembled in 1972 from records of persons who were exposed as early as 1955 (17 years prior) raises the likelihood that in 1972 a "survivor" population remained (45 deaths before 1972 were eliminated from the cohort) that was relatively healthy. Furthermore, the unusually large number of not "natural" expected and observed deaths (probably accidents and external causes) occurring to this cohort indicate a relatively youthful population was under scrutiny. The leading cause of death to persons under 35 years is from accidents, based on national vital statistics.

The authors correctly note that, because of limitations in the study material, only powerful carcinogenic effects could be detected. Risk ratios higher than 1.5 for all cancers, 4.0 for lymphomas and 10.0 for STS could be excluded based on this data set from the authors own calculations. More follow-up is needed in order to provide a stable assessment of the relationship between exposure and cancer. The authors concluded that this study will allow no assessment of STS because "the number of persons having a sufficiently long latency period is too small." It was suggested that more valid conclusions could be made only with the passage of time (Riihimaki et al., 1983).

Recently, the Michigan Department of Public Health (1983b), conducted an ecological study of soft and connective tissue cancer mortality rates in Midland and other selected Michigan counties. They found that mortality rates for this cause were 3.8-4.0 times the national average for the periods 1960-1969 and 1970-1978, respectively, for white females in Midland. These estimates are based upon 5 deaths and 7 deaths, respectively, and are listed in Table VI-5. No excess risk was reported among white males, however. The Michigan Department of Health concluded that because of the occurrence of these two successive elevated rates, it is unlikely to be a chance happening. At the same time the age-adjusted male and female cancer mortality rates for Midland were below that of the State of Michigan for the period

TABLE VI-5

Midland County Soft and Connective Tissue Cancer Deaths 1960-1981*

Identification			Type of Malignancy			
Year of Death	Sex	Age	Type	Primary Site	Metastases	Month and Year Diagnosed
1961	F	24	Hemangiosarcoma	Face	Skull and upper lobe of lung	5-58
1963	F	75	Liposarcoma	Right gluteal	Unknown	Unknown
1964	F	51	Leiomyosarcoma	Uterus	Widespread	11-63
1968	F	37	Liposarcoma	Spine	Lungs, pelvis	1-66
1969	F	45	Fibrosarcoma Leiomyosarcoma	Right thigh Uterus	Lung, liver Adrenal gland and skin	10-68
1970	F	59	Kaposi sarcoma	Right leg	Lymph nodes	8-68
1970	F	56	Fibrosarcoma Leiomyosarcoma	Right thigh Abdominal wall	Spine Lung	1960 1967
1974	F	1	Rhabdomyosarcoma	Inguinal area	Unknown	8-73
1976	F	77	Liposarcoma	Right thigh	Buttock, lung, rib, lymph nodes	12-74
1978	F	64	Leiomyosarcoma	Left knee	Liver, lymph nodes, lung, bone	7-70

TABLE VI-5 (cont.)

Identification			Type of Malignancy			
Year of Death	Sex	Age	Type	Primary Site	Metastases	Month and Year Diagnosed
1978	F	26	Rhabdomyosarcoma	Rectum	Lung, neck, inguinal region	6-76
1978	F	88	Fibrosarcoma	Right cheek	Facial area	6-78
1979	F	27	Leiomyosarcoma	Left thigh	Lung	3-78
1962	M	63	Rhabdomyosarcoma	Left lower leg	Lung and right outer chest wall	8-61
1967	M	77	Mesothelioma	Lung	Lung, peritoneum and diaphragm	6-67
1967	M	20	Rhabdomyosarcoma	Pharynx	Periorbital area and liver	1-67
1969	M	32	Liposarcoma	Left arm	Perineum and buttock	6-64
1971	M	76	Leiomyosarcoma	Small intestine	Liver	10-69
1972	M	89	Leiomyosarcoma	Retro-peritonal region	Hepatic system	7-72
1976	M	53	Fibrosarcoma	Peritioneum	Lung, liver	3-75

*Source: Michigan Department of Public Health, 1983b

1970-1979. Midland County is the home of a major chemical company that produced phenoxyacetic acid herbicides until recently. The authors stated that a detailed review of death certificates, hospital records, residency and occupational histories of the 20 male and female cases revealed no "commonalities" suggesting a "single causative agent," although a majority or their spouses had worked at this chemical facility. They recommend that a case-control study should be instituted to evaluate possible influences, such as lifestyle, occupation or location of residence on the risk of STS.

In a separate review of the epidemiological evidence for STS from exposure to 2,4,5-T-containing herbicides, the United Kingdom Ministry of Agriculture, Fisheries and Food (1983) concluded that there was no evidence to recommend altering their earlier conclusion that formulations of phenoxy acid herbicides and related wood preservatives as "presently cleared" are safe and may continue to be used. This report too readily discounts the positive studies of Hardell and Eriksson (1981) as being biased, and it makes no reference to the later validity study by Hardell (1981) of his own work utilizing colon cancer controls. In this report Hardell effectively answered these early criticisms that were reiterated by the British in their report. At the same time, the British report appears to put undue emphasis on nonpositive studies that do not demonstrate a risk, although most of them have methodological limitations (e.g., low power, insufficient latency and inappropriate study method). In short, the British review appears to be overly optimistic about the safety of 2,4,5-T herbicides.

In summary, the associations reported in the two Swedish soft-tissue sarcoma studies are great enough to make it unlikely that they have resulted entirely from random variation bias or confounding, even though the possibility cannot be excluded. These studies provide a strong suggestion that phenoxyacetic acid herbicides, chlorophenols or their impurities are carcinogenic in humans.

A separate series of clinical observations at the Department of Oncology in Umea, Sweden (Hardell, 1979), led the researchers to conduct a case-control study of malignant lymphoma in relation to phenoxyacetic acid, chlorophenols, and other organic compounds (Hardell et al., 1980, 1981). Approximately 33% of the cases in this study were patients with Hodgkin's disease; the remainder of the cases were non-Hodgkin's lymphomas.

This study employed essentially the same methods and produced results comparable with those of the STS studies: statistically significant 5-fold to 6-fold relative risks in relation to phenoxyacetic acids and chlorophenols. In addition, an elevated relative risk was found in connection with exposure to organic solvents, such as benzene, trichloroethylene and styrene. In the published report, the methods and results were incompletely documented, especially the possibility of confounding by exposure to the organic solvents.

In the update of the earlier 1980 study, Hardell et al. (1981), utilizing the same basic data source, found that 36.1% of the cases had been exposed to phenoxy herbicides or chlorophenols, while only 9.6% of their controls were so exposed. The estimated relative risk was 6.0 when matching was considered and 5.3 when matching was eliminated. When cases and controls who were exposed to chlorophenols only were excluded, the relative risk of lymphoma from phenoxy acids alone was 4.8 (95% C.I. 2.9-8.1). On the other hand, if exposures to phenoxy acids are excluded and consideration is given to just chlorophenols (which includes combined exposure to phenoxy acids and chlorophenols), then the relative risk equaled 4.3 (95% C.I. 2.7-6.9). The authors further subdivided this group into "low-grade" vs. "high-grade" exposures to chlorophenols. A continuous exposure of not more than 1 week or repeated intermittent exposures totaling not more than 1 month was classified as low-grade. The relative risk for high-grade exposure was 8.4 (95% C.I. 4.2-16.9), while that for low-grade exposure equaled 9.2 (95% C.I. 1.6-5.2). If exposure to organic solvents is examined, given that cases and controls exposed to only phenoxy acids or chlorophenols or both were excluded except for combined exposure to organic solvents, it is found that highgrade and low-grade relative risks were 2.8 (95% C.I. 1.6-4.8) and 1.2 (95% C.I. 0.5-2.6), respectively. However, the authors noted that exposure to phenoxy acids and high-grade organic solvents (exposure to chlorophenols excluded) produced a relative risk of 11.2 (95% C.I. 3.2-39.7) based upon a few cases and controls with exposure to both. The authors concluded that "exposure to organic solvents, chlorophenols or phenoxy acids constitutes a risk factor for malignant lymphoma."

This latter study is still subject to the same methodological criticisms to which the earlier study was subjected. Chief among those is the possibility of observational or recall bias creeping into the responses that are elicited from self-administered questionnaires on kind and length of exposure. Secondly, confounding by exposure to potentially carcinogenic organic solvents and other agents could have had an effect on the risk estimate, although the authors assure the reader that they did not (Hardell et al., 1981).

Other research has tentatively suggested that lumberjacks may be at increased risk of lymphoma (Edling and Granstam, 1979). The Nitro study found three deaths from cancers of the lymphatic and hematopoietic system, against only 0.88 expected (p=0.06, one-tailed Poisson test).

The lymphoma case-control study (Hardell et al., 1980, 1981) is consistent with the two STS studies discussed. On the other hand, the consistency could also reflect an as-yet unidentified consistent flaw in all these studies.

The two Swedish case control studies on STSs and a later case control study of malignant lymphoma (Hardell et al., 1981) were subjected to a validity analysis with respect to the assessment of exposure by Hardell and Eriksson (1981). To answer the question raised regarding the recall of occupation in a forestry/agriculture job, secondary to the recall of exposure to phenoxy acids or chlorophenols or both, the cases and controls were divided into three groups: those who worked their entire time since 1950 in an agriculture/forestry job, those who worked some time in an agriculture/forestry job but not exclusively, and the remainder who never worked in a forestry/agriculture job. The study found that the risk ratio was still 8.2 for STS in exclusively agriculture/forestry workers who were exposed to phenoxy acids compared with workers found in other occupations having no apparent exposure to phenoxy acids or chlorophenols. Even when comparing phenoxy acid or chlorophenol exposed agricultural/forestry workers exclusively with nonexposed agricultural/forestry workers, the risk ratio was still 7.1. This argument seems to effectively answer questions regarding recall of occupation secondary to exposure.

On the other hand, the relative risk remains 5.4 when comparing phenoxy acid or chlorophenol exposed workers exclusively in occupations other than agriculture/forestry with nonexposed workers in those same occupations, thus suggesting the presence of either recall bias or still another occupation with potential exposure to phenoxy acids or chlorophenols (Table VI-6).

When woodworkers are separated out (possible exposure to chlorophenols in treatment of wood) the risk ratio becomes 9.7 (Table VI-7). These data suggest the presence of some recall bias.

Another focus of (Hardell and Eriksson, 1981) this study was to determine if observational bias on the part of the investigators could explain the significantly high risk estimates. To answer the question, the study compared the exposure data derived from the interviewee's returned questionnaires only with the combined information from both the phone interviews and questionnaires. The study found no substantial differences in the frequency of reporting exposure.

Still a third consideration of possible bias involves recall of exposure to phenoxy acids or chlorophenols because of subject knowledge of having cancer in the cases versus no knowledge of cancer in the referent population. The study chose as a referent group for the 52 STS cases (Hardell and Sandstrom, 1979) and the 169 malignant lymphomas (Hardell et al., 1981) a group of 154 colon cancer cases from the same population source and compared their exposure to phenoxy acids or chlorophenols by broad age groupings and by rural versus urban residence.

TABLE VI-6

Other Occupations (Minus Forestry/Agriculture)*

Group	Phenoxy Acids/Chlorophenols	Non-exposed
Cases	11	68
Referents	5	167
	RR = 5.4	$X^2 = 11.01$ ($p<0.01$)

*Source: Adapted from Hardell and Erikkson, 1981

RR = relative risk

TABLE VI-7

Other Occupations (Minus Forestry/Agriculture/Woodworkers)*

Group	Phenoxy Acids/Chlorophenols	Non-exposed
Cases	4	66
Referents	1	160
	RR = 9.7	$X^2 = 5.98$ ($p<0.05$)

*Source: Adapted from Hardell and Erikkson, 1981

RR = relative risk

Utilizing a Mantel-Haenszel rate ratio, the study found the risk of exposure to phenoxy acids remaining significantly high at 5.5 and to chlorophenols 5.4 in the STS cases compared with the colon cancer controls. Similarly, with the malignant lymphomas, the identically derived risk ratios remain significantly high at 4.5 with respect to phenoxy acids or chlorophenol exposure in the cases; hence, the study concludes that, no "substantial observational bias" exists. If the study is assuming that recall bias was and is the same as observational bias, then such a conclusion may not be entirely warranted from the comparison. Certainly, it appears that no recall bias existed because of subject "knowledge of having cancer" based on the authors analysis. But it does not rule out the possibility that recall bias can still be present in their data for other reasons. Hardell and Eriksson (1981) refer to an intense "debate about phenoxy acids and their presumptive risk" in Sweden at the time the colon cancer study was conducted. But, there is no reason to think that colon cancer victims would assume their disease was brought about from exposure to dioxin containing chemicals if no connection was suggested.

It seems plausible that STS and non-Hodgkin's lymphoma patients would either learn at the time of their diagnosis that exposure to dioxin containing chemicals was the likely cause of this rare type of tumor or quickly learn from other sources, such as the news media, that exposure to herbicides containing dioxin could cause their rare form of cancer. Whereas, colon cancer victims (a rather common form of cancer) would not necessarily be led to believe that exposure to the same dioxin containing chemicals caused their disease. Hence, it is not difficult to imagine that such unusual victims of cancer could better "remember" exposure to such chemicals than could colon cancer patients.

Therefore, although this study by Hardell and Eriksson (1981) may explain any biases introduced from secondary recall of occupation, observational bias introduced from the telephone interviewer and recall bias based on subject knowledge of cancer, it does not adequately answer questions of recall bias introduced through the acquired awareness on the part of the victim of STS or non-Hodgkin's lymphoma that his condition may have been caused by exposure to dioxin containing herbicides.

Studies of two of the oldest cohorts of workers known to have been exposed to phenoxyacetic acid herbicides or 2,3,7,8-TCDD or both report stomach cancer mortality rates significantly higher than expected. The results in each study were based on small numbers of deaths. In one study (Axelson et al., 1980), 348 Swedish railroad workers with at least 46 days of herbicide exposure between 1955 and 1972 were followed through October 1978. The workers were grouped on the basis of their primary herbicide exposures: those primarily exposed to phenoxyacetic acids (2,4-D and 2,4,5-T) only, to amitrole (aminotriazole) only, and to both types of herbicides. After a 10-year latency was achieved, 3 stomach cancer deaths were observed vs. 0.71 expected ($p<0.05$). None were attributable to amitrol alone, but two were assigned to phenoxy acids alone while the remaining stomach cancer death occurred in a worker exposed to both amitrol and phenoxy acids in combination. The excess was more pronounced (3 observed vs. 0.57 expected, $p<0.05$) among those with early exposure (1957-1961) to phenoxy acids or amitrol or both. If persons who were exposed to just amitrol alone are excluded, thus leaving individuals exposed to phenoxy acid alone and amitrol in combination, the excess is enhanced further (3 observed vs. 0.41 expected, $p<0.01$).

Axelson et al. (1980) also note an excess in total "tumors" after 10 years latency as well (15 observed vs. 6.87 expected, $p<0.005$). This is pronounced in those exposed early to phenoxy acids alone (6 observed vs. 2.60 expected, $p<0.01$) and phenoxy acids in combination with amitrol (5 observed vs. 1.34 expected, $p<0.05$). Presumably, "tumors" in Sweden are analogous to malignant neoplasms in the United States. The author states that no specific type of tumor predominates and no breakdown by tumor type is provided.

The other study showing increased stomach cancer mortality is the follow-up of 75 workers exposed to 2,3,7,8-TCDD during and after a 1953 run-away reaction at a trichlorophenol manufacturing facility in Ludwigshafen, Federal Republic of Germany (Theiss and Frentzel-Beyme, 1977). Two sources were used to calculate expected deaths: national mortality rates for the period 1971-1974, and 1972-1975 rates for Rhinehessen-Palatinate, the region in which Ludwigshafen is located.*

The results, shown in Table VI-8, indicate an increased rate of stomach cancer mortality that also is not likely to have been due to chance alone.

Two aspects of the methodology used should be noted that could have influenced these results. First, the available report does not include an analysis allowing for a minimum period of cancer induction. All three

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*The report originally included expected deaths using rates for the city of Ludwigshafen, which were later shown to be inaccurate.

TABLE VI-8

Analysis of Stomach Cancer Mortality in a Group of West German Factory Workers Exposed to 2,3,7,8-TCDD*

Source for Expected Deaths	Stomach Cancer Deaths		Relative Risk	Significance Level
	Observed	Expected		
Federal Republic of Germany 1971-1974	3	0.559	5.4	0.02
Rhinehessen-Palatinate 1972-1975	3	0.495	6.1	0.01

*Source: Adapted from Theiss and Frentzel-Beyme, 1977 stomach cancer deaths in the Ludwigshafen cohort occurred more than 10 years after initial exposure. Employing a 10-year restriction to follow-up (as in the Swedish cohort study) would result in a higher relative risk estimate by reducing the number of expected deaths.

Secondly, national and regional mortality rates from the 1970s were used to generate expected deaths to compare with observed mortality over a much longer period (1953-1977). Strong temporal trends in stomach cancer mortality in West Germany during the late 1950s and 1960s would make these expected figures too large.

The researchers also used an internal control group that does not raise the second concern discussed above. This group consisted of 75 men, each matched to study group members by age and date of entry into employment, and selected at random from a list of over 10,000 persons who had been included in previous cohort studies by the same investigators. No stomach cancer deaths occurred in this control group during the follow-up period. Thus, use of the internal control groups also indicates an excess of stomach cancers in the exposed workers.

In an update of this earlier study, Theiss et al. (1982) continued the follow-up of his cohort through 1979 by adding 2 additional years of follow-up and apparently reducing the size of his cohort from 75 to 74. Altogether 21 deaths (4 more than from the earlier study) occurred vs. 18 and 19 deaths in the 2 matched (1 to 1) internal comparison groups. With respect to cancer deaths, the numbers were respectively 7, 5 and 5. The first control group was manually matched from the total number of persons (5500 included in the cohort until the end of 1976) and the second, at random, by computer for some 8000 employees. In addition, 19 expected total deaths were estimated based on 1970-1975 mortality statistics of Rhinehessin-Palatinate, 18 expected deaths based on 1970-1975 mortality statistics of Ludwigshafen, and 20 expected deaths based upon 1971-1974 mortality statistics of the Federal Republic of Germany. Just as in the earlier study, the three stomach carcinomas noted earlier appear to be significantly elevated regardless of which external comparison group is used (Table VI-9).

On the other hand, one stomach cancer appeared in the randomized internal control group. None appeared in the manually matched internal control. No other elevated risks for any other cause were evident and no STSs appeared. When latency was considered only, the risk of stomach cancer remained significantly elevated after a lapse of 10 years (3 observed, 0.52 expected, p<.016) and then after a lapse of 15 years (2 observed, 0.23 expected, p<.02) based upon death rates of Rhinehessin-Palatinate, 1970-1975.

Again, these study conclusions are limited by the small size of the study group and the very few cancer deaths noted at any particular site. Thus, it is insensitive to the detection of a significantly elevated risk for most causes of cancer, especially STS and lymphomas. Although, stomach cancer is elevated significantly, it is based only upon three deaths and since one stomach cancer death has been noted in an internal control group in the updated version, it appears that this finding has been weakened somewhat. Furthermore, as was pointed out earlier, trends in stomach cancer mortality during the 1950s, 1960s and 1970s could make the comparison of stomach cancer mortality with expected deaths less valid based upon 1970-1975 rates.

TABLE VI-9

Reanalysis of Stomach Cancer Mortality in a Group of West German Factory Workers Exposed to 2,3,7,8-TCDD*

Source for Expected Deaths	Stomach Cancer Deaths		Relative Risk	Significance Level
	Observed	Expected		
Federal Republic of Germany 1971-1974	3	0.7	4.3	0.034
Rhinehessin-Palatinate 1970-1975	3	0.64	4.7	0.027
Ludwigsshafen 1970-1975	3	0.61	4.9	0.024

*Source: Adapted from Theiss et al., 1982

In summary, the evidence that phenoxyacetic acids or 2,3,7,8-TCDD or both might increase the risk of stomach cancer consists of two studies, each of which reports a statistically significant excess that is based on only three stomach cancer deaths. Further follow-up of these and similar cohorts is warranted, but firm conclusions cannot yet be made.

Four additional cohort studies have reported results that do not show increased stomach cancer mortality rates in groups of workers exposed to phenoxyacetic acids or 2,3,7,8-TCDD or both. These are studies of 2,4,5-T production workers in Midland, Michigan (Ott et al., 1980), Finnish phenoxyacetic acid herbicide applicators (Riihimaki et al., 1978), the Nitro study in which workers were exposed to 2,3,7,8-TCDD (Zack and Suskind, 1980) and trichlorophenol manufacturing workers (Cook et al., 1980).

As previously mentioned, the Nitro study included a single death from STS and a weakly suggestive increase in lymphatic and hematopoietic system cancer mortality. The Midland study included only one cancer death, a tumor in the respiratory system. In the Finnish study, histologic information on tumor types was not provided; however, there were no deaths from lymphoma.

The results pertinent to stomach cancer mortality in the three studies are shown in Table VI-10. Neither the Midland study nor the Nitro study contradicts the findings of the Swedish and West German investigations previously discussed. This can be shown in two ways. First, the upper 95% confidence limits for the relative risk estimates from these two "negative" studies exceed even the highest point estimates of relative risk (6.1) from the two "positive" studies (see Table VI-8).

TABLE VI-10

Stomach Cancer Mortality in Three Studies of Workers Exposed to Phenoxyacetic Acid Herbicides and/or 2,3,7,8-TCDD

Stomach Cancer Deaths		Relative Risk	95% Confidence Interval	Reference
Observed	Expected			
0	0.14[a]	0	0-26.3	Ott et al., 1980
5	6.9[a,b]	0.7	0.2-1.7	Riihimaki et al., 1978
0	0.5[b]	0	0-7.4	Zack and Suskind, 1980

[a] Estimated from total cancer expected deaths (see footnote in text).

[b] Entire follow-up period without regard for minimum time for cancer induction (Ott et al., 1980 used a 10-year minimum induction period).

This indicates that the relative risk estimates from the Midland and Nitro studies, even though equal to zero, are nevertheless not significantly different from the estimates of 6.1, given the sample sizes, follow-up periods, age distribution and comparison group rates.

In addition, the smallest detectable relative risk in the Midland study ($\alpha = 0.05$, $\varphi = 0.2$ one-tailed Poisson test) was 21.4 (3 observed deaths, 0.14 expected).* Similarly, the smallest detectable relative risk in the Nitro study ($\alpha = 0.05$, $\varphi = 0.2$, one-tailed Poisson test) was 10.0 (5 observed deaths, 0.5 expected). This calculation is based on results for the entire follow-up period. If, as in the Midland study, a minimum period of cancer induction had been employed, the expected deaths would have been fewer and the smallest reasonably detectable relative risk would have been greater. This analysis of statistical power indicates that the Nitro and Midland studies had very low probabilities of detecting the ~6-fold increases in risk suggested by the Swedish and West German investigations.

Statistically, the study of Finnish herbicide applicators is inconsistent with the results of the Swedish and West German cohort studies. The smallest reasonably detectable relative risk ($\alpha = 0.05$, $\varphi = 0.2$, one-tailed Poisson test) was only 3.1 (11 observed deaths, 3.6 expected).* The study, therefore, appears powerful enough to detect relative risks even smaller than those seen in the Swedish and West German studies. A partial explanation for this apparent inconsistency could lie in the fact that the Finnish study set the minimum period of herbicide exposure for membership in the cohort at 10 days (2 working weeks) and noted that the "total strength of exposure has, in most cases, been a few weeks only." The Swedish study of herbicide applicators set the minimum exposure at 46 days (>1 spraying season).

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*Ott et al. (1980) did not report expected deaths from stomach cancers. The figure 0.14 was obtained by multiplying the numbers of expected deaths from all cancers (2.6, allowing a 10-year minimum induction period) by the percentage of stomach cancers among the expected deaths in the Nitro study (0.5/9.04 = 5.5%). The two studies used United States white male mortality rates and covered similar calendar years in follow-up (1949-1978 in Nitro and 1950-1976 in Midland), but a similarity in age distributions cannot be established from the published reports.

There are also certain inconsistencies in the data from the Finnish study that the authors note but find difficult to explain. In particular, no cancer deaths occurred during the latter part of the study period among Forestry Authority workers (1 of 4 groups included in the cohort), even though 9.0 deaths were expected. This finding strongly suggests some deficiency in follow-up or in the source records from which vital status was determined.

In summary, four cohort studies of workers exposed to phenoxyacetic acid herbicides or 2,3,7,8-TCDD or both do not report increased risks of stomach cancer. Only one of these, however, was statistically powerful enough to be inconsistent with the two studies that tentatively suggest an increase in stomach cancer risk. The available report of this study of Finnish herbicide applicators contains methodologic questions that require clarification.

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*The expected stomach cancer deaths were estimated in the same manner as for the Midland study. A proportion of 20% of all cancer deaths was applied because Finnish male mortality rates are known to be very high.

By adding together the number of workers exposed to phenoxy acids or chlorophenols or both from all case studies, an unusually high number of STSs is shown, considering the rarity of the disease. This excess is suggestive of an association of cancer with exposure to phenoxy acids or chlorophenols or both, and consequently, with the impurities found in these herbicides, including 2,3,7,8-TCDD.

Two Swedish case-control studies report highly significant association of STS with exposure to phenoxy acid or chlorophenols or both. They do not pinpoint the risk to the dioxin contaminants, however. In fact, in one study, the risk was found to extend to phenoxy acids free of dioxin impurities. In that study, the risk increases to 17 when phenoxy acids known to contain dioxin impurities (polychlorinated dibenzodioxins and dibenzofurans) are considered. The extent of possible observer bias and recall bias introduced into these studies by using self-administered questionnaires is not of sufficient magnitude to have produced the highly significant risks found in the studies.

Later studies did not reveal a significant excess risk of STS. However, methodology problems make these latter studies limited with respect to evaluating the risk of STSs from exposure to phenoxy acids or chlorophenols or both and, consequently, 2,3,7,8-TCDD.

The Swedish case-control studies provide limited evidence for the carcinogenicity of phenoxy acids or chlorophenols or both in humans. However, with respect to the dioxin impurities contained therein, the evidence for the human carcinogenicity for 2,3,7,8-TCDD based on the epidemiologic studies is only suggestive because of the difficulty of evaluating the risk of 2,3,7,8-TCDD exposure in the presence of the confounding effects of phenoxy acids and/or chlorophenol.

There is less evidence incriminating 2,4,5-T or 2,3,7,8-TCDD or both as the cause of malignant lymphoma and stomach cancer in humans.

High Risk Subpopulations

Little information was found in the available literature to indicate that specific human subpopulations may be unusually susceptible to the toxic effects of 2,3,7,8-TCDD. Limited evidence from the Seveso area and Eastern Missouri indicates that children may be more sensitive to the immunological effects of 2,3,7,8-TCDD. Tognoni and Bonaccorsi (1982) found elevated peripheral blood lymphocytes, lymphocyte blastogenic response, and serum complement activity in exposed children; however, no immunological effects were detected in adults (Reggiani, 1980; May, 1982). Among adults and children exposed to contaminated soil in horse arenas in Eastern Missouri, the only adverse health effects reported were in children (Kimbrough et al., 1977). Children playing in the arena were probably in more intimate contact with the contaminated soil, and thus subject to higher exposures, than were the adults.

Summary

By adding together the number of workers exposed to phenoxy acids and/or chlorophenols from all case studies, an unusually high number of STSs is shown, considering the rarity of the disease. This excess is suggestive of an association of cancer with exposure to phenoxy acids and/or chlorophenols, and consequently, with the impurities found in these herbicides, including 2,3,7,8-TCDD.

Two Swedish case-control studies report highly significant association of STS with exposure to phenoxy acid and/or chlorophenols. They do not pinpoint the risk to the dioxin contaminants, however. In fact, in one study, the risk was found to extend to phenoxy acids free of dioxin impurities. In that study, the risk increases to 17 when phenoxy acids known to contain dioxin impurities (polychlorinated dibenzodioxins and dibenzofurans) are considered. The extent of possible observer bias and recall bias introduced into these studies by using self-administered questionnaires is not of sufficient magnitude to have produced the highly significant risks found in the studies.

Later studies did not reveal a signficant excess of risk of STS. However, methodology problems make these latter studies limited with respect to evaluating the risk of STSs from exposure to phenoxy acids and/or chlorophenols and, consequently, 2,3,7,8-TCDD. Epidemiological studies on more recently exposed populations, where dioxin exposure can be more accurately assessed, and subsequent studies, including a higher number of deaths, on populations previously studied will increase the pool of data on dioxin and help evaluate its carcinogenic risk in humans.

Either acute or chronic exposure to 2,3,7,8-TCDD may result in chloracne, altered liver function, hematological pathologies, porphyria cutanea tarda, hyperpigmentation, hirsutism and neural degeneration in the extremities. Stevens (1981) has estimated that the minimum cumulative toxic dose of 2,3,7,8-TCDD in humans is 0.1 µg/kg.

The toxic effects of exposure to 2,3,7,8-TCDD may persist for many years, through some effects seem to be reversible in some cases. Even though 2,3,7,8-TCDD has been found to be fetotoxic and/or teratogenic in all animal species tested (see the Teratogenicity and Reproductive Toxicity Section in Chapter V), epidemiological studies have failed to demonstrate a convincing connection between 2,3,7,8-TCDD exposure and spontaneous abortions or malformations in humans. These studies are difficult to interpret, since quantitative exposure data are not available. Some evidence of cytogenetic damage has been reported in humans exposed to chemicals contaminated with 2,3,7,8-TCDD, but negative results have also been reported; exposures were not quantitated; and the other chemicals cannot be ruled out as causative agents.

VII. MECHANISM OF TOXICITY

A number of studies have attempted to determine the mechanism of toxicity of 2,3,7,8-TCDD. The ultimate purpose is to provide a better estimate of man's relative sensitivity to 2,3,7,8-TCDD and other compounds having a similar mode of action. Specifically, these studies may be able to explain the reason for the marked interspecies differences in 2,3,7,8-TCDD toxicity and, thus, help determine if humans possess factors that are associated with sensitivity to 2,3,7,8-TCDD toxicity.

Receptor-Mediated Toxicity

Pharmacogenetic studies have played an important role in understanding the biologic and toxic effects of drugs and xenobiotics. Nebert and coworkers have shown that carcinogenic polycyclic aromatic hydrocarbons (PAHs) induce the cytochrome P-450-dependent monooxygenase AHH in certain responsive strains of mice (e.g., C57Bl/6J, BALBc, C3HF/He) whereas this PAH induction activity is minimal or nonexistent in nonresponsive strains (DBA/2J) (Nebert, 1979, 1982; Nebert and Gielen, 1972; Nebert and Jensen, 1979; Nebert et al., 1972, 1981, 1983). The gene complex responsible for the induction of AHH and several other enzymes has been designated the Ah locus which comprises regulatory, structural and possible temporal genes. Extensive studies on genetically inbred responsive and nonresponsive mice (and their backcrosses) indicate that these differences are related to the Ah regulatory gene and its gene product, the Ah cytosolic receptor protein. This receptor protein interacts with PAH ligands and the resultant PAH:Ah receptor complex translocates into the nucleus and presumably initiates the induction of AHH via a process comparable to that proposed for the steroid hormones.

Since the carcinogenic and toxic effects of PAHs are dependent on their oxidative metabolism to reactive electrophilic forms, it is not surprising that the Ah receptor plays an important role in mediating their toxicity and carcinogenicity (Kouri, 1976; Kouri et al., 1974; Benedict et al., 1973; Shum et al., 1979; Thomas et al., 1973; Legraverend et al., 1980; Duran-Reynolds et al., 1978; Robinson et al., 1975; Mattison and Thorgeirsson, 1979). Responsive mice are more susceptible to the toxic (inflammation, fetotoxicity, primordial oocyte depletion) and carcinogenic effects of PAH at organs/tissues in direct contact with the applied chemical; in contrast, nonresponsive mice are more susceptible to the tumorigenic effects of PAHs at tissue/organ sites remote from the initial site of exposure to the PAHs. These differences in susceptibility are due to several factors including AHH-mediated toxication and detoxication.

2,3,7,8-TCDD: Segregation of Activity with the Ah Locus. Genetic studies also support the role of the Ah receptor in mediating the toxic and biologic effects of 2,3,7,8-TCDD. Initial studies by Poland and coworkers (Poland et al., 1974, 1983; Poland and Glover, 1975; Nebert et al., 1975) demonstrated that the microsomal AHH-inducing activity of 2,3,7,8-TCDD and 3-MC in several genetically inbred mice strains were similar. Like 3-MC and related PAHs, 2,3,7,8-TCDD induced AHH in several responsive mouse strains (i.e., C57Bl/6J). In contrast to 3-MC, 2,3,7,8-TCDD induced microsomal AHH in the DBA/2J nonresponsive mice; however, the ED_{50} for this biologic response was significantly higher than values reported for the responsive mice. In genetic crosses between responsive C57Bl/6 and nonresponsive DBA/2 mice it was also shown for both 3-MC and 2,3,7,8-TCDD that the trait of responsiveness is inherited in a simple autosomal dominant mode (Poland and

Knutson, 1982). It has been suggested that the observed differences in the activities of 3-MC and 2,3,7,8-TCDD are related to their relative Ah receptor affinities (Poland and Knutson, 1982) and the pharmacokinetic and metabolic factors which would more rapidly diminish the "available" concentrations of 3-MC caused by metabolism and excretion.

Several studies with 2,3,7,8-TCDD in genetically inbred mice support the receptor mediated hypothesis. The induction of UDP-glucuranosyl transferase, DT diaphorase, δ-aminolevulinic acid, glutathione-S-transferase B, T-aldehyde dehydrogenase and cholinekinase by 2,3,7,8-TCDD or 3-MC in genetically inbred mice have also been shown to segregate with the Ah locus (Beatty and Neal, 1976b; Owens, 1977; Kirsch et al., 1975; Dietrich et al., 1978; Ishidate et al., 1980; Poland and Glover, 1973a). Toxicology studies with genetically-inbred mice confirm the role of the Ah locus in mediating several toxic effects including porphyria, immunotoxicity (a wasting syndrome), thymic atrophy and cleft palate formation (Jones and Sweeney, 1980; Poland and Glover, 1980; Courtney and Moore, 1971; Vecchi et al., 1980, 1983). Poland et al. (1982) have also linked the tumor-promoting activity of 2,3,7,8-TCDD in hairless mice to the cytosolic receptor. In vitro studies with XB cells in culture also support the role of receptor in mediating a dose-related cell keratinization by 2,3,7,8-TCDD which resembles some of the characteristics of chloracne (Knutson and Poland, 1980). This cell line is also responsive to AHH induction and contains a cytosolic receptor binding protein. Although the murine Ah receptor has not been characterized, several studies confirm that a protein with high affinity for 3-MC and 2,3,7,8-TCDD is present in low concentrations in the hepatic (~30-50 fmolar) and extrahepatic tissues of responsive C57Bl/6J mice

(Greenlee and Poland, 1979; Okey et al., 1979, 1980; Poland et al., 1976; Mason and Okey, 1982; Gasiewicz and Neal, 1982; Okey and Vella, 1982; Okey, 1983; Nebert et al., 1983). In responsive C57Bl/6J mice and Sprague-Dawley rats, but not in nonresponsive DBA/2J mice, the Ah receptor can be induced by pretreatment with phenobarbital which is the only known agent at present that has been demonstrated to affect tissue concentrations of the receptor (Okey and Vella, 1984). Although the Ah receptor has not been detected in the cytosol of DBA/2J mice, after the administration of radiolabeled 2,3,7,8-TCDD to these mice, some of the radiolabel is detected in the nuclei of the nonresponsive mice. Moreover, the sedimentation characteristics of the [^{3}H]-2,3,7,8-TCDD:nuclear protein complex in DBA/2J mice are similar to those observed with the bound Ah cytosolic receptor protein in C57Bl/6J mice using a sucrose density gradient centrifugation separation technique (Okey, 1983). Several reports have also demonstrated that the cytosolic Ah receptor protein migrates into the nucleus of the cell only after binding with 2,3,7,8-TCDD (Greenlee and Poland, 1979; Okey et al., 1979, 1980) and this parallels the observations noted for the interactions between steroids and their receptor proteins.

2,3,7,8-TCDD and Related Toxic Halogenated Aryl Hydrocarbons: Structure-Activity Correlations. The evidence for a receptor mediated mechanism of action for 2,3,7,8-TCDD is supported by data reported for the effects of other halogenated aryl hydrocarbons in genetically inbred mice and other diverse animal species. A number of reviews and comparative studies (Allen et al., 1979; Allen and Norback, 1977; Kimbrough, 1974; Kimbrough et al., 1978; McConnell and Moore, 1979; Taylor, 1979) clearly indicate that the toxic halogenated mixtures and individual compounds (including the PCDDs, PCDFs, PCBs and PBBs) elicit similar toxic and biologic responses which include 1) a wasting syndrome which is manifested by a progressive weight loss and decreased food consumption by the treated animals; 2) skin disorders including acneform eruptions or chloracne, alopecia, edema, hyperkeratosis, and hypertrophy of the Meibomian glands; 3) lymphoid involution and atrophy; 4) porphyria (resembling porphyria cutanea tarda); 5) endocrine and reproductive disorders; 6) modulation of chemical carcinogenesis; and 7) the induction of numerous enzymes including the cytochrome P-448 (or P-450c) dependent monooxygenases. It is apparent that the effects of these compounds are not manifested in all the animal species tested. McConnell and Moore (1979) summarized the pathologic findings observed in several animal species after pretreatment with PCDDs, PCDFs, PCBs and PBBs and these data illustrate the different species and organ/tissue susceptibilities to these compounds. It is also evident that for most of these effects, all the toxic halogenated aromatics elicit similar effects in these species which also contain the cytosolic receptor protein (Carlstedt-Duke, 1979; Carlstedt-Duke et al., 1979, 1981; Okey, 1983; Okey and Vella, 1982; Mason and Okey, 1982). These observations support a common mechanism of action for all the toxic halogenated aryl hydrocarbons (Poland and Knutson, 1982; Safe et al., 1982; McConnell and Moore, 1979).

Several reports have demonstrated the effects of structure on the activity of PCDDs. The most active member of this group is substituted in the lateral 2, 3, 7 and 8 positions; activity is decreased with 1) decreasing lateral substituents, and 2) increasing Cl substitution. Moreover, for several PCDDs, there is an excellent correlation between the toxicity of individual PCDD congeners in guinea pigs and mice (McConnell et al., 1978b) and their AHH induction potencies in chick embryos and rat hepatoma H-4-II-E cells in culture and their binding affinities for the C57Bl/6J mouse hepatic cytosolic receptor protein (Poland et al., 1976, 1979; Bradlaw et al., 1980; Bradlaw and Casterline, 1979). Comparable structure-activity correlations have been reported for the PCDFs in which the most active compound, 2,3,7,8-TCDF, is an approximate isostereomer of 2,3,7,8-TCDD (Poland et al., 1979; Poland and Knutson, 1982). Moreover, like the PCDDs, there was an excellent correlation between the toxicity of several individual PCDFs (Yoshihara et al., 1981), their AHH induction potencies in rat H-4-II-E hepatoma cells and binding affinities to male Wistar rat hepatic cytosolic receptor protein (Bandiera et al., 1983).

The most active PCB congeners, 3,4,4',5-tetra-, 3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl, are substituted at both para and at two or more meta positions. The four coplanar PCBs induce rat hepatic microsomal AHH and cytochromes P-450a, P-450c and P-450d and resemble 3-MC and 2,3,7,8-TCDD in their mode of induction of the cytochrome P-450 isozymes (34) (Parkinson et al., 1980a,b, 1983; Safe et al., 1982; Sawyer and Safe, 1982; Poland and Glover, 1977; Goldstein et al., 1977). Like Aroclor 1254, all the monoortho and at least eight diortho-chloro analogs of the coplanar PCBs exhibited a "mixed-type" induction pattern and induced microsomal AHH, DMAP N-demethylase and cytochromes P-450a to P-450e (Parkinson et al., 1980a,c, 1983). Quantitative structure-activity relationships (QSARs) within this series of PCBs were determined by comparing their AHH induction potencies (EC_{50}) in rat hepatoma H-4-II-E cells and their binding affinities (ED_{50}) for the 2,3,7,8-TCDD rat cytosolic receptor protein (Sawyer and Safe, 1982; Bandiera et al., 1983). The results showed that there was an excellent correlation between AHH induction potencies and receptor binding avidities of these compounds and the order or activity was coplanar PCBs (3,3',4,4'-tetra-, 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyls) > 3,4,4',5-tetrachlorobiphenyl > mono-*ortho* coplanar PCBs > di*ortho* coplanar PCBs. It was also apparent that the relative toxicities of this group of PCBs paralleled their biological potencies (Biocca et al., 1981; Yoshihara et al., 1979; Marks et al., 1981; McKinney et al., 1976; Yamamoto et al., 1976; Ax and Hansen, 1975; Kuroki and Masuda, 1977).

The coplanar and mono*ortho* coplanar PCBs also exhibit differential effects in the inbred C57Bl/6J and DBA/2J mice. These compounds induce AHH and cause thymic atrophy in the former "responsive" mice whereas at comparable or higher doses none of these effects are observed in the nonresponsive DBA/2J mice (Parkinson et al., 1982; Robertson et al., 1984). The results obtained for structurally diverse PCDDs, PCBs and PCDFs clearly support the role of the receptor protein in initiating the broad spectrum of biologic and toxic effects elicited by these chemicals. Bandiera et al. (1983) have demonstrated that the 2,3,7,8-TCDD receptor protein is not only susceptible to halogen substitution patterns but also the structure of the substituent. The cytosol receptor binding avidities and AHH induction potencies in rat hepatoma H-4-II-E cells for several 4'-X-2,3,4,5-tetrachlorobiphenyls were remarkably dependent on the structure of the X substituent. The binding data for 13 different substituents was subjected to multiparameter regression analysis to correlate binding avidities with the physical chemical characteristics of the critical lateral X substituents. The equation

$$\log \left(\frac{1}{EC_{50}}\right) = 1.53\sigma + 1.47\,\pi + 1.09\,HB + 4.08$$

showed that ligand binding was dependent on substituent electronegativity (σ), lipophilicity (π) and hydrogen binding (HB) with a correlation coefficient (r) equal to 0.978 for 13 different substituents.

The receptor mediated hypothesis for the mechanism of action of 2,3,7,8-TCDD still requires further confirmation and numerous problems must be clarified. For example:

1. Several cell culture lines which appear to have the Ah receptor are highly resistant to the toxicity of 2,3,7,8-TCDD; the nonresponsive HTC and responsive H-4-II-E cell lines (i.e., for AHH inducibility by 2,3,7,8-TCDD) do not possess cytosolic receptor; however, the nonresponsive HTC cells possess more nuclear receptor binding protein than the responsive H-4-II-E cells (Okey, 1983; Okey et al., 1980).
2. Hepatic cytosolic receptor levels in rats (Wistar and Sprague-Dawley), C57Bl/6J mice, hamsters and guinea pigs are comparable (Gasiewicz et al., 1983b); however, their susceptibility to the biologic and toxic effects of 2,3,7,8-TCDD are highly variable: guinea pigs are highly susceptible to the lethal effects of 2,3,7,8-TCDD (LD_{50} = 1-2 μg/kg) whereas the susceptibility of the other species follows the order rat > C57Bl/6J mice > DBA/2J mice > hamster (Neal et al., 1982).

Metabolism

The metabolism of 2,3,7,8-TCDD has been examined in the guinea pig, rat, mouse and hamster. Urine and bile from ^{14}C-TCDD-treated animals were found to be free of unmetabolized 2,3,7,8-TCDD, demonstrating that metabolism was required for elimination through these routes (Olson et al., 1983). The direct intestinal elimination of unchanged 2,3,7,8-TCDD in feces suggests, however, that some routes of excretion may not be dependent on prior metabolism of the toxin (Olson et al., 1983). Thus, it is not possible to directly correlate the half-life for elimination of 2,3,7,8-TCDD with its in vivo rate of metabolism in a given species. The relative persistence of 2,3,7,8-TCDD in a given species may be related to the in vivo rate of 2,3,7,8-TCDD metabolism, excretion of the toxin not dependent upon metabolism (direct intestinal elimination, lactation, sebum), and the relative tissue distribution of 2,3,7,8-TCDD, particularly to adipose stores. Qualitative and quantitative differences in the metabolism and disposition of 2,3,7,8-TCDD have been observed between various species, and these may in part be related to the remarkable interspecies differences in sensitivity to 2,3,7,8-TCDD toxicity (Olson et al., 1983).

Poiger et al. (1982a) suggest that 2,3,7,8-TCDD metabolism represents detoxification, since they observed relatively little toxicity in guinea pigs given extracts of dog bile containing 2,3,7,8-TCDD metabolites. However, a recent study proposes that metabolites of 2,3,7,8-TCDD may inhibit uroporphyrinogen decarboxylase activity and lead to 2,3,7,8-TCDD-induced porphyria (DeVerneuil et al., 1983). Current data on the structural identification of 2,3,7,8-TCDD metabolites suggest that reactive epoxide intermediates may be formed during metabolism (Poiger et al., 1982b; Sawahata et al., 1982). Poland and Glover (1979) reported that the maximum possible *in vivo* covalent binding of 1,6-^{3}H-2,3,7,8-TCDD derived radioactivity to hepatic DNA was 4 orders of magnitude less than the levels of binding observed with other chemical carcinogens. The study did find much higher levels of 2,3,7,8-TCDD derived radioactivity bound to hepatic protein of the rat. No data is available, however, on the degree 2,3,7,8-TCDD derived radioactivity is bound to tissues of various species of laboratory animals, which have demonstrated remarkable variability in sensitivity to 2,3,7,8-TCDD. While biliary excretion products may represent detoxified, polar metabolites of 2,3,7,8-TCDD, it remains to be shown whether unexcreted reactive metabolites initiate some of the toxic responses associated with exposure to this toxin.

Vitamin A Depletion

Many of the toxic effects of 2,3,7,8-TCDD resemble the effects of vitamin A deficiency, such as epithelial lesions, keratosis and immunosuppression. The administration of a single oral dose of 0.1, 1.0 or 10 µg 2,3,7,8-TCDD/kg bw produces a dose-related decrease in the hepatic storage of retinol in Sprague-Dawley rats (Thunburg et al., 1979, 1980). The authors suggested, but did not demonstrate, that the low storage of retinol in the 2,3,7,8- TCDD-treated animals is the result of an increased turnover of retinol. These results suggest that an induced vitamin A deficiency may be responsible for some, but not all, of the toxic effects produced by 2,3,7,8-TCDD. At the highest dose of 2,3,7,8-TCDD, dietary retinol supplements could not fully compensate for the 2,3,7,8-TCDD-produced decrease in hepatic retinol content.

Lipid Peroxidation

Increased lipid peroxidation has been suggested as a possible mechanism of 2,3,7,8-TCDD-induced toxicity (Sweeney and Jones, 1983). This hypothesis is based on the following limited pieces of evidence. First, iron deficiency inhibits _in vitro_ lipid peroxidation (Bus and Gibson, 1979; Sweeney et al., 1979; and reduces the hepatotoxic effects of 2,3,7,8-TCDD (Sweeney et al., 1979). Secondly, lipofuscin pigments, by-products of lipid peroxidation, are increased in the heart muscle of rats treated with 2,3,7,8-TCDD (Albro et al., 1978). Thirdly, Sweeney and Jones (1983) reported that administration of the antioxidant butylated hydroxyanisole (BHA) at a level of 0.75% in the diet provided some protection from 2,3,7,8-TCDD-induced prophyria and neutral lipid accumulation. At this dose level of BHA, 4 of the 6 mice (sex not specified) tested were protected; however, at a lower dose (0.25%), all animals were protected from these toxic effects. No beneficial effects were observed when the antioxidant vitamin E (0.01%) was included in the diet.

Recently, Stohs et al. (1983) obtained direct evidence that 2,3,7,8-TCDD accelerates lipid peroxidation in Sprague-Dawley rats. Groups of 4-8 female rats were treated for 3 days with 2,3,7,8-TCDD at doses of 0, 10, 20 or 40 µg/kg by gavage (in a corn oil vehicle). At days 1, 6 and 11 after the last treatment the animals were sacrificed and lipid peroxidation was determined in isolated liver microsomes by the reaction of formed malondialdehyde with thiobarbituric acid. At all sacrifice periods, increased lipid peroxidation was observed and the increase was dose-related. The maximal increase detected on day 6 after the last treatment was 5- to 6-fold greater than in the controls. In addition, these workers measured lipid peroxidation in vivo by the determination of conjugated dienes in rats receiving 2,3,7,8-TCDD at 40 µg/kg. Using this latter method, similar increases in lipid peroxidation were detected, although the maximal increase of 2.35-fold was observed at day 1 postexposure rather than day 6. The authors suggested that the in vivo formation of reactive free radicals during lipid peroxidation could account for the nonspecific nature of 2,3,7,8-TCDD toxicity.

Endocrine Imbalance

Some of the toxic response to 2,3,7,8-TCDD, including hirsutism and diminishing libido, indicate that 2,3,7,8-TCDD may produce some of its toxicity through endocrine disturbances (Oliver, 1975). Nienstedt et al. (1979) reported that a single oral dose of 20 µg 2,3,7,8-TCDD/kg bw significantly reduced testosterone catabolism. Catabolism of exogenous estrogen in ovariectomized rats is also decreased by 2,3,7,8-TCDD pretreatment (Shiverick and Muther, 1982). In this study, there was a 57% increase in serum estrone concentrations following administration of 10 mg estrone/100 g bw/day for 4 days to either control or 2,3,7,8-TCDD pretreated ovariectomized rats. No differences were observed in the increase in uterine wet weight following estrone administration in control and 2,3,7,8-TCDD pretreated rats. Thus, the uterotrophic response was not altered by any 2,3,7,8-TCDD-mediated change in estrone disposition.

Shiverick and Muther (1983) also measured estradiol metabolism in female Holtzman rats given 2,3,7,8-TCDD at a dose of 1 μg/kg bw on days 4-19 of gestation. At this fetal toxic dose, the catechol estrogen formation ability of isolated liver microsomes from the dams was decreased 50% when measured on day 20 of gestation. These microsome preparations had a 4-fold increase in the 7α-hydroxylation of testosterone, while there was no change in the 16α- or 6β-hydroxylase activity. Although steroid metabolism was altered in microsomes isolated from 2,3,7,8-TCDD-treated pregnant rats, similar exposure of pregnant rats on days 4-15 of gestation did not result in any change in circulating levels of serum 17β-estradiol. The authors suggested that other mechanisms besides liver metabolism of steroids may be involved in the fetotoxic effect of 2,3,7,8-TCDD.

Gustafsson and Ingelmar-Sundberg (1979) observed that 2,3,7,8-TCDD produced greater change in steroid metabolism in female Sprague-Dawley rats than in male rats of the same strain, resulting in a liver enzyme pattern displaying less sex differentiation than in uninduced rats. Based on this result, they propose that some of the effects of 2,3,7,8-TCDD result from an interaction with the hypothalamo-pituitary axis, rather than from a direct effect on steroid metabolism.

Since glucocorticoid hormones are known to have a catabolic effect on lymphoid tissues, such as the thymus and spleen, and these tissues degenerate after exposure of rats to 2,3,7,8-TCDD, Neal et al. (1979) investigated the ability of 2,3,7,8-TCDD to either stimulate the production or mimic the effects of these hormones. In male Sprague-Dawley rats treated by gavage with 2,3,7,8-TCDD at a dose of 50 μg/kg (the ~LD_{50}), there was a slight depression in blood glucocorticoids during post-treatment days 1-4, followed by an ~2.5-fold increase on post-treatment days 7 and 14. While in competitive binding assays between 2,3,7,8-TCDD and a synthetic hormone, dexamethasone, 2,3,7,8-TCDD had no affinity for the hormone receptor. Thus, 2,3,7,8-TCDD may stimulate glucocorticoid production, but was not able to mimic the action of these hormones by binding to the glucocorticoid receptor. It was determined, however, that the increase in glucocorticoids was likely not to participate in the toxicity of 2,3,7,8-TCDD through adrenal hyperfunction, since prior adrenalectomy did not provide any protection from the lethal effects of 2,3,7,8-TCDD in rats.

Summary

Carcinogenic PAHs induce cytochrome P-450-dependent monooxgenase AHH in genetically responsive strains of mice, possibly by binding to a cytosolic receptor protein that translocates to the nucleus to initiate induction of AHH (Nebert, 1979, 1982; Nebert and Gielen, 1972; Nebert and Jensen, 1979; Nebert et al., 1972, 1981, 1983). Since the carcinogenic and toxic effects of PAHs require oxidation to reactive electrophils, it is likely that the Ah receptor and AHH induction play an important role in mediating their toxicity and carcinogenicity.

Several studies with 2,3,7,8-TCDD in inbred mice support the Ah receptor mediated hypothesis and have shown that effects associated with the Ah receptor (porphyria, immunotoxicity, thymic atrophy, cleft palate) segregate with the Ah locus (Beatty and Neal, 1976b; Poland and Glover, 1973a, 1980; Jones and Sweeney, 1980). In responsive mice and Sprague-Dawley rats, the Ah receptor can be induced with phenobarbital, which has been demonstrated to increase the concentration of the receptor.

A number of reviews and comparative studies (Allen et al., 1979; Allen and Norback, 1977; Kimbrough, 1974; Kimbrough et al., 1978; McConnell and Moore, 1979; Taylor, 1979) indicate that the toxic halogenated compounds (PCDDs, PCDFs, PCBs and PBBs) all elicit similar toxic responses (a wasting syndrome, skin disorder, lymphoid atrophy and immunodeficiency, porphyria, endocrine and reproductive disorders, modification of chemical carcinogenesis and hepatic enzyme induction) in several different species. A common cytosolic receptor protein appears to be present in species that respond similarly to these compounds, indicating a common mechanism of action (Safe, 1982; McConnell and Moore, 1979; Poland and Knutson, 1982).

Structure activity studies have determined that the 2,3,7,8-(laterally substituted) member is the most active of the PCDDs (McConnell et al., 1978b). Of the PCDFs, 2,3,7,8-TCDF is the most active (Poland et al., 1979). Activity of the toxic halogenated aryl hydrocarbons correlates well with their ability to induce AHH in chick embryos (McConnell et al., 1978b) and rat hepatomas H-4-II-E cells (McConnell et al., 1978b; Yoshihara et al., 1981). The activity of the various PCDD congeners has also been correlated with their binding affinity with cytosolic receptor protein in mouse (Poland et al., 1976, 1979; Bradlow et al., 1980; Bradlow and Casterline, 1979) or rat (Bandiera et al., 1983) hepatic cytosolic receptor protein. Activity of PCDD congeners is diminished by decreasing lateral substitution or increasing Cl substitution in the other positions (McConnell et al., 1978b).

Slight toxicity was observed in guinea pigs administered extracts of the bile of dogs treated with 2,3,7,8-TCDD led Poiger et al. (1982a) to conclude that metabolism represents detoxification. The structural identification of 2,3,7,8-TCDD metabolites, however, has led investigators (Poiger et al., 1982b; Sawahata et al., 1982) to hypothesize that reactive epoxide intermediates may form that account for a substantial amount of the toxicity associated with 2,3,7,8-TCDD. Compared to other chemical carcinogens, however, the binding of 1,6-^{3}H-2,3,7,8-TCDD derived radioactivity to hepatic DNA was less by about four-orders of magnitude than the carcinogen DMN (Poland and Glover, 1979). Much higher levels of radioactivity, however, were found bound to cytosolic protein.

Since some of the effects of 2,3,7,8-TCDD toxicity resemble vitamin A deficiency (epithelial keratosis, immunosuppression) Thunburg et al. (1979, 1980) investigated the ability of single low oral doses of 2,3,7,8-TCDD to reduce hepatic storage of retinol in rats. A dose-related decrease in hepatic retinol was demonstrated. At the highest dose of 2,3,7,8-TCDD, dietary retinol supplements could not fully compensate for the 2,3,7,8-TCDD-induced hepatic losses of retinol.

Increased lipid peroxidation has been suggested as a possible mechanism for 2,3,7,8-TCDD-induced toxicity (Sweeney and Jones, 1983). It was demonstrated that iron deficiency, which reduces lipid peroxidation, reduced 2,3,7,8-TCDD-induced hepatotoxicity (Bus and Gibson, 1979; Sweeney et al., 1979). Lipofuscin pigments, by-products of lipid peroxidation, accumulate in the cardiac muscle of 2,3,7,8-TCDD treated rats (Albro et al., 1978). Butylated hydroxyanisole, a known antioxidant, provided some protection to mice treated with 2,3,7,8-TCDD (Sweeney and Jones, 1983). Dietary vitamin E was not protective in this study. Recently, a dose-related increase in liver microsomes was demonstrated in rats treated with oral doses of 2,3,7,8-TCDD (Stohs et al., 1983).

Some of the effects associated with 2,3,7,8-TCDD (hirsutism, diminished libido) suggest that the compound may induce some of its toxicity through endocrine disturbances (Oliver, 1975). 2,3,7,8-TCDD has been shown to retard catabolism of testosterone (Nienstedt et al., 1979) and estrone (Shiverick and Muther, 1982) and estradiol (Shiverick and Muther, 1983).

Since glucocorticoids are known to have a lytic effect on lymphoid tissues and lymphoid atrophy is part of the picture of 2,3,7,8-TCDD toxicity, Neal et al. (1979) investigated the ability of the compound to stimulate corticoid production or mimic its effect by competitively binding to receptor sites. Rats treated with a single dose of 2,3,7,8-TCDD responded with slightly decreased blood corticoids for ~4 days followed by a substantial (~2.5-fold) increase on post-treatment days 7-14. 2,3,7,8-TCDD did not, however, bind to receptor sites to which dexamethasone did bind. Gustafsson and Ingelman-Sundberg (1979) proposed a hypothalamic-pituitary axis for the action of 2,3,7,8-TCDD on steroid levels rather than a direct effect on steroid metabolism.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the noncarcinogenic and carcinogenic effects. In the quantification of noncarcinogenic effects, an acceptable daily intake (ADI) is calculated. An adjusted acceptable daily intake (AADI) and health advisory (HA) values for the chemical are then calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$\text{ADI} = \frac{\text{(NOAEL or LOAEL in mg/kg/day) (body weight in kg)}}{\text{Uncertainty/Safety Factor}} = \text{mg/day}$$

$$\text{AADI} = \frac{\text{ADI}}{\text{Drinking water volume in } \ell\text{/day}} = \text{mg}/\ell$$

where:

NOAEL = no-observed-adverse-effect level
LOAEL = lowest-observed-adverse-effect level
Body weight = 70 kg for adult or 10 kg for child
Drinking water volume = 2 ℓ/day for adults or 1 ℓ/day for children
Uncertainty/Safety Factor = 10, 100 or 1000

Utilizing these equations, the following drinking water concentrations are developed for noncarcinogenic effects:

1. A 1-day HA for 10 kg child
2. A 1-day HA for 70 kg adult
3. A 10-day HA for 10 kg child
4. A 10-day HA for 70 kg adult
5. A lifetime AADI for a 70 kg adult

The distinctions made between the HA calculations (items 1-4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited period of exposure (possibly 1-2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon noncarcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivities to toxic chemicals. An Office of Drinking Water (ODW) modification of the guidelines set forth by the National Academy of Sciences (NAS, 1977, 1980) are typically used in establishing uncertainty factors as follows:

- An uncertainty factor of 10 is used when good acute or chronic human exposure data are available and supported by acute or chronic toxicity data in other species.
- An uncertainty factor of 100 is used when good acute or chronic toxicity data identifying NOEL/NOAEL are available for one or more species, but human data are not available.
- An uncertainty factor of 1000 is used when limited or incomplete acute or chronic toxicity data are available or when only the acute or chronic toxicity data identify a LOAEL (but not NOEL/NOAEL) for one or more species are available.

The uncertainty factor used for a specific risk assessment is judgmental. Factors that cannot be incorporated in the NAS/ODW guidelines (NRC, 1977) for selection of an uncertainty factor, but must be considered, include the following: 1) the quality of the toxicology data, 2) the significance of the adverse effect, and 3) the existence of counterbalancing beneficial effects.

If toxicological evidence requires the chemical to be classified as a potential carcinogen mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical by drinking water. The bioassay data used in these estimates are from animal experiments. In order to predict the risk for humans, these data must be converted to an equivalent human dose. This conversion includes correction for noncontinuous animal feeding, nonlifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 ℓ of water/day. The multistage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The estimates of low doses for these models can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as 1) incomplete knowledge concerning the health effects of contaminants in drinking water, 2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study results and 3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

The U.S. EPA has established the limits of 1.3×10^{-7}, 1.3×10^{-8} or 1.3×10^{-9} µg 2,3,7,8-TCDD/ℓ in ambient waters, based on an assumed daily consumption of 6.5 g of contaminated fish and shellfish and 2 ℓ of drinking water (U.S. EPA, 1984a). Under these conditions, 94.2% of the total exposure would result from the consumption of aquatic organisms. The recommended levels correspond to estimated human lifetime excess cancer risks of 10^{-5}, 10^{-6} or 10^{-7}, respectively. These values are considerably lower than the HAs for drinking water that are recommended in this document (see the Summary Section of this chapter), reflecting the high bioaccumulation of this compound in aquatic species.

The FDA advises that fish containing >50 ppt of 2,3,7,8-TCDD should not be consumed and those containing >25 ppt, but <50 ppt, should not be consumed more than twice a month (FDA, 1983). This is reflected in a Canadian limit of 20 ppt in the Lake Ontario commercial fish imported into the United States (NRCC, 1981).

An ADI of 10^{-4} μg 2,3,7,8-TCDD/kg bw/day has previously been proposed by the National Academy of Sciences Committee on Drinking Water and Health (NAS, 1977). This ADI was based on a 13-week rat feeding study by Kociba et al. (1976) and was proposed before convincing evidence for the carcinogenicity of 2,3,7,8-TCDD had accumulated.

Noncarcinogenic Effects

The characteristic effects of exposure to 2,3,7,8-TCDD include thymic atrophy and weight loss (see the General Toxicity Section in Chapter V). In rats and rabbits, and to a lesser extent in guinea pigs and monkeys, liver damage is a major pathological symptom. Death is commonly preceded by a prolonged period of weight loss, during which time severe deterioration of the animals is observed; however, no specific lesion has been identified as the cause of death. Death generally occurs 1-7 weeks following an acute exposure. This unusual characteristic of 2,3,7,8-TCDD toxicity has resulted in many short-term studies that report only minor effects at doses near, or sometimes many-fold greater that the LD_{50}. 2,3,7,8-TCDD is also an immunosuppressant in mice, rats and guinea pigs.

The acute toxicity of 2,3,7,8-TCDD varies among species tested. Acute oral LD_{50}s ranging from 0.6 μg/kg bw for male guinea pigs to 5051 μg/kg bw for hamsters have been reported (see Table V-1). The relative sensitivity of man to 2,3,7,8-TCDD toxicity, compared with other species, cannot be determined from the existing data.

1-Day HA. The data used for the determination of a 1-day HA are summarized in Table VIII-1. LD_{50} data, while not useful in the derivation of an HA, are a useful way to compare species susceptibility and are, therefore, included in the table for comparison purposes. Four studies were found that identified NOAELs or LOAELs that could be useful in the derivation of an HA (Harris et al., 1973; Madge, 1977; Smith et al., 1981; Turner and Collins, 1983).

Harris et al. (1973) administered a single oral dose of 2,3,7,8-TCDD in acetone:corn oil to groups of CD rats of mixed sex. Weights were determined at least once each week. Rats given 50 or 100 μg/kg bw demonstrated a decreased weight gain and increased mortality. In the high dose group, mortality approached 50% with a mean time interval until death of 18.3 days. A dose of 25 μg/kg bw, the LOAEL in this study, resulted in a decreased body weight in females at 1 week postdosing and a decreased rate of weight gain in males for 2 weeks postdosings. After 2 weeks, both male and female rats gained weight at the same rate as the controls. Doses of 1 or 5 μg/kg bw had no effect on body weight.

Madge (1977) investigated the effect of 2,3,7,8-TCDD on intestinal absorption in CD-1 mice. Mice were given single oral doses of 10, 25, 75, 150, 200 or 300 μg 2,3,7,8-TCDD/kg bw. Absorption of D-glucose, D-galactose, L-argenine and L-histidine was measured 7 days later, using the

TABLE VIII-1

Acute Toxicity of 2,3,7,8-TCDD

Species	Route of Exposure	Dose μg/kg/day	Duration of Exposure	Duration of Experiment	Effect Level	Endpoints	Reference
Rat	oral	25	1 day	8-9 weeks	LOAEL	Decreased body weight	Harris et al., 1973
Rat (male)	oral	22.0	1 day	2-8 weeks	LD_{50}		Schwetz et al., 1973
Rat (female)	oral	45.0	1 day	2-8 weeks	LD_{50}		Schwetz et al., 1973
Rat	i.p.	2.5	1 day	8 weeks	LOEL	Increased serum triglycerides	Poli et al., 1980
Mouse	i.p.	1	1 day	NA	LOAEL	Decreased macrophage and natural killer cell number	Mantovani et al., 1980
Mouse	oral	10	1 day	7 days	LOAEL	Decreased intestinal absorption of d-glucose	Madge, 1977
Mouse	oral	50	1 day	12 weeks	LOAEL	Porphyria	Smith et al., 1981
Mouse	oral	15	1 day	12 weeks	NOAEL	Porphyria	Smith et al., 1981
Mouse	oral	284	1 day	30 days	LD_{50}		McConnell et al., 1978a
Mouse	oral	114	1 day	2 months	LD_{50}		Vos et al., 1974
Guinea pig (female)	oral	0.1	1 day	42 days	LOAEL	Mild histopathologic affects on liver	Turner and Collins, 1983
Guinea pig (male)	oral	0.6	1 day	2-8 weeks	LD_{50}		Schwetz et al., 1973
Guinea pig (female)	oral	2.1	1 day	2-8 weeks	LD_{50}		Schwetz et al., 1973
Guinea pig (male)	oral	2.0	1 day	30 days	LD_{50}		McConnell et al., 1978a
Rabbit	oral	115	1 day	2-8 weeks	LD_{50}		Schwetz et al., 1973
Rabbit	dermal	275	1 day	3 weeks	LD_{50}		Schwetz et al., 1973
Hamster	oral	5051	1 day	55-71 days	LD_{50}		Henck et al., 1981
Hamster	oral	1157	1 day	50 days	LD_{50}		Olson et al., 1980b
Hamster	i.p.	>3000	1 day	50 days	LD_{50}		Olson et al., 1980b
Monkey	oral	<70	1 day	>35 days	LD_{50}		McConnell et al., 1978b

i.p. = Intraperitoneal

everted intestinal sac technique. Absorption of D-glucose was decreased at all dose levels; however, absorption of the other compounds was not affected by any of the treatment. The decrease in D-glucose absorption was dose-related over the range of 0-75 µg/kg bw. In this study, 10 µg/kg bw constituted a LOAEL.

Smith et al. (1981) investigated the effect of 2,3,7,8-TCDD on hepatic porphyrin levels in C57Bl/10 and DBA/2 mice. A single oral dose was administered in arachis oil (0, 5, 15, 50, 75, 150, 300, 600 or 1200 µg/kg bw) and hepatic porphyrin levels were determined at intervals for up to 12 weeks. There were large strain differences in susceptibility to porphyria induction, with the C57Bl/10 strain being ~20 times as sensitive as the DBA/2 strain. In this study, the lowest dose which induced porphyria was 50 µg/kg bw. Thus, 50 µg/kg bw was a LOAEL and 15 µg/kg bw represented a NOAEL.

Turner and Collins (1983) administered single oral doses of 0.1, 0.5, 2.5, 12.5 or 20 µg/kg bw of 2,3,7,8-TCDD in aqueous methyl cellulose to groups of 4-6 female guinea pigs. Survivors were killed 42 days after dosing and examined for histopathologic changes in the liver. Four of the 6 animals in the highest dose group and 1 of 5 in the 12.5 µg/kg group died before the end of the observation period. Mild histopathologic changes including steatosies (fatty change), focal necrosis and cytoplasmic degeneration were noted in animals from all treated groups, but not in controls. The authors indicated that qualitative differences among the dosage groups were not detectable by light microscopy.

All of the LOAELs and NOAELs determined for rats and mice are above the LD_{50} for guinea pigs (0.6-2.1 μg/kg). Although no NOEL or NOAEL is available for guinea pigs, a LOAEL of 0.1 μg/kg can be derived from the study of Turner and Collins (1983).

10-Day HA. Studies which can possibly be used for calculation of a 10-day HA are identified in Table VIII-2. However, the study by Turner and Collins (1983) used for the calculation of the 1-day HA is proposed as the basis for calculation of a 10-day HA. By dividing the 1-day HA by 10 to convert the 1-day HA to 10-day HA, a 10,000-fold uncertainty factor is, in effect, applied to the 0.1 μg/kg LOAEL in the Turner and Collins (1983) study. The calculation of a 10-day HA on this basis is concluded to be consistent with 10-day HA calculations which could be considered using the data in Table VIII-2 and applying uncertainty factors of 1000 and 100 to LOAEL and NOAEL data as appropriate.

Long-Term Exposure. The health effects of long-term exposure to 2,3,7,8-TCDD are summarized in Table VIII-3. Most of the long-term studies have been performed using rats. There is, therefore, only limited information on the chronic toxicity of 2,3,7,8-TCDD in other species. Of the studies included in Table VIII-3, only those by Kociba et al. (1978a,b, 1979) and Murray et al. (1979) were done with administration of 2,3,7,8-TCDD in the diet on a daily basis.

TABLE VIII-2

Effects of 4-13 Weeks Exposure to 2,3,7,8-TCDD

Species	Route of Exposure	Dose µg/kg	No. of Treatments/ Week	Duration of Exposure	Effect Level	Endpoints	Reference
Rat	oral	0.01	5	13 weeks	NOAEL	Decreased body weight	Kociba et al., 1976
Rat	oral	0.1	5	13 weeks	LOAEL	Decreased body weight	Kociba et al., 1976
Rat	oral	0.5	2	13 weeks	NOAEL	Toxic hepatitis	NTP, 1980a
Rat	oral	1.0	2	13 weeks	LOAEL	Toxic hepatitis	NTP, 1980a
Rat	oral	0.1	7	30 days	LOAEL	Decreased thymus weight	Harris et al., 1973
Rat	oral	1.0	1	6 weeks	NOAEL	Decreased body weight	Harris et al., 1973
Rat	oral	0.1	7	30 days	LOAEL	Thrombocytopenia	Zinkl et al., 1973
Rat	oral	5.0	1	6 weeks	LOAEL	Decreased body weight and thymus weight	Vos et al., 1973
Rat	oral	1.0	1	6 weeks	NOAEL	Decreased body weight and thymus weight	Vos et al., 1973
Rat	oral	0.001	7	3 generation	LOAEL	Decreased body weight, decreased fertility, decreased fetal survival	Murray et al., 1979
Mouse	oral	1.0	2	13 weeks	LOAEL	Toxic hepatitis	NTP, 1980a
Mouse	oral	5.0	1	4 weeks	NOAEL	Porphyria	Goldstein et al., 1978
Mouse	oral	25.0	1	4 weeks	LOAEL	Porphyria	Goldstein et al., 1978
Mouse	oral	5.0	1	4 weeks	LOAEL	Decreased thymus weight and graft-versus-host response	Vos et al., 1973
Mouse	oral	1.0	1	4 weeks	NOAEL	Decreased thymus weight and graft-versus-host response	Vos et al., 1973
Mouse	oral	1.0	1	4 weeks	LOAEL	Decreased resistance to Salmonella	Thigpen et al., 1975
Mouse	oral	1.5	1	4 weeks	LOAEL	Increased endotoxic (E. coli) susceptibility	Vos et al., 1978a
Mouse	oral	10 ppb	7	5 weeks	LOAEL	Decreased tetanus response, antigenic RBC response, sensitization to DNFB, resistance to Salmonella infection, resistance to Listeria infection	Hinsdill et al., 1980

TABLE VIII-2 (cont.)

Species	Route of Exposure	Dose µg/kg	No. of Treatments/ Week	Duration of Exposure	Effect Level	Endpoints	Reference
Mouse	i.p.	0.4	1	4 weeks	LOAEL	Decreased cytotoxic T-cell response	Clark et al., 1981
Guinea pig	oral	0.008	1	8 weeks	NOAEL	Thymus weight and tuberculin hypersensitivity	Vos et al., 1973
Guinea pig	oral	0.04	1	8 weeks	LOAEL	Thymus weight and tuberculin hypersensitivity	Vos et al., 1973

i.p. = Intraperitoneal

TABLE VIII-3

Effects of Long-Term Exposure to 2,3,7,8-TCDD

Species	Route of Exposure	Dose µg/kg	No. of Treatments/ Week	Duration of Exposure	Effect Level	Endpoints	Reference
Rat	oral	0.001	7	3 generation	LOAEL	Decreased body weight, decreased fertility, decreased fetal survival	Murray et al., 1979;
Rat	oral	0.01	1	16 weeks	NOAEL	Elevated porphyrin levels	Goldstein et al., 1982b
Rat	oral	0.1	1	16 weeks	LOAEL	Elevated porphyrin levels	Goldstein et al., 1982b
Rat	oral	0.1	2	28 weeks	LOAEL	Fatty changes in liver, decreased body weight	King and Roesler, 1974
Rat	oral	0.001	7	104 weeks	NOAEL	Degenerative and necrotic changes in the liver	Kociba et al., 1978a,b, 1979
Rat	oral	0.01	7	104 weeks	LOAEL	Degenerative and necrotic changes in the liver	Kociba et al., 1978a,b, 1979
Rat	oral	0.01	2	104 weeks	NOAEL	Toxic hepatitis	NTP, 1980a
Rat	oral	0.05	2	104 weeks	LOAEL	Toxic hepatitis	NTP, 1980a
Rat	oral	1.0	1	45 weeks	LOAEL	Porphyria	Cantoni et al., 1981
Rat	oral	0.01	1	45 weeks	LOEL	Hepatic enzyme induction, liver weight	Cantoni et al., 1981
Mouse	oral	0.01	2	104 weeks	LOAEL	Toxic hepatitis	NTP, 1980a
Mouse	oral	0.007	1	52 weeks exposure, 104 weeks study duration	LOAEL	Dermatitis and amyloidosis	Toth et al., 1978, 1979

The EPA has developed an ADI based on noncarcinogenic effects as indicated in U.S. EPA (1984a). For consistency, the rationale used by EPA for the ADI calculation in U.S. EPA (1984a) is used for the ADI calculation herein. The rationale as presented in U.S. EPA (1984a) is as follows:

> 2,3,7,8-TCDD displays an unusually high degree of reproductive toxicity. It is teratogenic, fetotoxic and reduces fertility. In a 3-generation reproductive study, Murray et al. (1979) reported a reduction in fertility after daily dosing at 0.1 or 0.01 μg 2,3,7,8-TCDD/kg in the F_1 and F_2 generations of Sprague-Dawley rats. Although Murray et al. (1979) considered the lowest dose tested, 0.001 μg/kg, to be a no-observed-effect level (NOEL), a re-evaluation of these data by Nisbet and Paxton (1982), using different statistical methods, indicated that there was a reduction in the gestation index, decreased fetal weight, increased liver to body weight ratio, and increased incidence of dilated renal pelvis at the 0.001 μg/kg dose. The re-evaluated data would suggest that equivocal adverse effects were seen at the lowest dose (0.001 μg/kg/day) and that this dose should, therefore, represent a lowest-observed-adverse-effect level (LOAEL). Schantz et al. (1979) found reductions in fertility and various other toxic effects in rhesus monkeys fed a 50 ppt 2,3,7,8-TCDD diet for 20 months. This corresponds to a calculated daily dose of 0.0015 μg 2,3,7,8-TCDD/kg/day. These results suggest that monkeys may be somewhat more sensitive than rats, since the effects in monkeys were more severe and not equivocal. Since the data from the limited study by Schantz et al. (1979) are supportive of the findings by Murray et al. (1979), it seems reasonable to determine an ADI based on the LOAEL.

Quantification of Noncarcinogenic Effects

Calculation of 1-Day HA. The data on very short-term exposures, 1-14 days, are sufficient for the derivation of an HA. As previously discussed in the 1-Day HA Section, the available studies establish LOAELs for rats and mice which are greater than the LD_{50} for guinea pigs. A NOAEL is not available for guinea pigs, but a LOAEL of 0.1 μg/kg can be derived from the study of Turner and Collins (1983). This LOAEL can be used to calculate a 1-day HA, using an uncertainty factor (UF) of 1000 for an animal LOAEL.

This UF consists of two 10-fold factors to account for both intra and interspecies variability to the toxicity of this chemical in lieu of chemical-specific data, and an additional 10-fold factor because the HA is based on a LOAEL and not a NOAEL. The UFs are based on previous guidelines (NAS, 1977; Federal Register, 1980).

$$HA = dose \times bw \div (UF \times wc)$$

where

bw = body weight, 70 kg for adult
10 kg for child

wc = water consumption, 2 ℓ/day for adult
1 ℓ/day for child

Thus, for a 70 kg adult the 10-day HA is as follows:

$$\text{1-day HA} = (0.1\ \mu g/kg\ bw/day \times 70\ kg\ bw) \div (1000 \times 2\ \ell/day) = 0.0035\ \mu g/\ell$$

This HA is equivalent to 0.0070 μg/day or 0.00010 μg/kg bw/day.

For a 10 kg child the 10-day HA is as follows:

$$\text{1-day HA} = (0.1\ \mu g/kg\ bw/day \times 10\ kg\ bw) \div (1000 \times 1\ \ell/day) = 0.0010\ \mu g/\ell$$

This HA is equivalent to 0.0010 μg/day or 0.00010 μg/kg bw/day.

Calculation of 10-Day HA. A 10-day HA is calculated by dividing the 1-day HA by 10 to convert the 1-day HA to a 10-day HA. Therefore, along with using an uncertainty factor of 1000 for an animal LOAEL (i.e., 10-fold for intra- and 10-fold for interspecies variability to the toxicity of a chemical in lieu of specific data, and an additional 10-fold because the estimate is based on a LOAEL rather than a NOAEL), a 10-day HA can be calculated from the LOAEL of 0.1 μg/kg/day reported by Turner and Collins (1983).

$$HA = (dose \times bw) \div (UF \times wc \times 10)$$

where

bw = body weight, 70 kg for adult
10 kg for child

wc = water consumption, 2 ℓ/day for adult
1 ℓ/day for child

10 = division by 10 to convert 1-day HA to 10-day HA

Thus, for a 70 kg adult the 10-day HA is as follows:

$$\text{10-day HA} = (0.001\ \mu g/kg\ bw/day \times 70\ kg\ bw) \div (1000 \times 2\ \ell/day)$$
$$= 0.00035\ \mu g/\ell$$

This HA is equivalent to 0.0007 μg/day or 0.0000099 μg/kg bw/day.

For a 10 kg child the 10-day HA is as follows:

$$\text{10-day HA} = (0.001\ \mu g/kg\ bw/day \times 10\ kg\ bw) \div (1000 \times 1\ \ell/day \times 10)$$
$$= 0.0001\ \mu g/\ell$$

This HA is equivalent to 0.0001 μg/day or 0.00001 μg/kg bw/day.

Quantification of Lifetime AADI. The studies included in Table VIII-3 are considered for calculation of an AADI. The AADI is based on a LOAEL of 0.001 μg/kg for reproductive effects in the 3-generation reproductive study in rats by Murray et al. (1979) along the rationale discussed in the Long-Term Exposure Section.

Using the LOAEL of 0.001 μg/kg bw/day, because the Murray et al. (1979) study suggested that dose of 0.001 μg/kg bw/day may be a LOAEL for reproductive effects, and dividing this by an uncertainty factor of 1000 for an animal LOAEL, a lifetime AADI can be calculated. This uncertainty factor is based on previous guidelines (NAS, 1977; Federal Register, 1980).

$$\text{Lifetime AADI} = (0.001\ \mu g/kg\ bw/day \times 70\ kg\ bw) \div (1000 \times 2\ \ell/day)$$
$$= 0.000035\ \mu g/\ell$$

This AADI is identical to the 10-day HA for adults and is equivalent to an ADI of 70 pg/day or 1.0 pg/kg bw/day. This ADI is the same as that estimated in the AWQC document for TCDD for comparison purposes to the criteria derived from carcinogenicity data (U.S. EPA, 1984a).

Carcinogenic Effects

A number of epidemiological studies have attempted to relate 2,3,7,8-TCDD exposure to human health effects (see the Epidemiological Studies Section). These studies are limited by small sample sizes, short follow-up period and exposure to multiple compounds. These epidemiological studies do not unequivocally establish a relationship between 2,3,7,8-TCDD and the development of tumors in humans, though an association has been suggested with soft-tissue sarcomas (Hardell and Sandstrom, 1979; Eriksson et al., 1979, 1981), lymphomas (Hardell et al., 1980, 1981) and stomach cancer (Axelson et al., 1980; Theiss and Frentzel-Beyme, 1977).

In comparison, a number of cancer bioassays have clearly demonstrated the carcinogenic potential of 2,3,7,8-TCDD in animals (Kociba et al., 1978a,b; Van Miller et al., 1977a,b; Toth et al., 1979; NTP, 1980a,b). These studies are summarized in Table VIII-4. Oral administration of 2,3,7,8-TCDD, either in the diet or by gavage, results in the production of hepatocellular carcinomas in female rats and both sexes of mice (Kociba et al., 1978a,b; NTP, 1980a; Toth et al., 1979). Follicular-cell adenomas of the thyroid have been observed in both male rats and female mice (NTP, 1980a). Various squamous cell carcinomas have also been reported in both sexes of rats (Kociba et al., 1978a,b).

TABLE VIII-4

Carcinogenicity Bioassays of 2,3,7,8-TCDD by Oral and Dermal Exposure

Species	Route	Dose Range	Duration of Treatment	Duration of Study	Animals/ Group	Tumor Types Reported	Reference
Rat (male)	gavage	0-0.5 µg/kg bw/week	104 weeks	105-107 weeks	50	Follicular-cell adenoma or carcinoma of the thyroid	NTP, 1980a
Rat (female)	gavage	0-0.5 µg/kg bw/week	104 weeks	105-107 weeks	50	Neoplastic nodule or hepatocellular carcinoma of the liver	NTP, 1980a
Rat (male)	diet	0-1000 ppb	78 weeks	95 weeks	10	All tumors	Van Miller et al., 1977a,b
Rat (male)	diet	0-0.1 µg/kg bw/day	105 weeks	105 weeks	50	Squamous cell carcinoma of the hard palate and the tongue, adenoma of the adrenal cortex	Kociba et al., 1978a,b
Rat (female)	diet	0-0.1 µg/kg bw/day	105 weeks	105 weeks	50	Hepatocellular carcinoma, squamous cell carcinoma of the tongue and lung	Kociba et al., 1978a,b
Mouse (male)	gavage	0-0.5 µg/kg bw/week	104 weeks	105-107 weeks	50	Hepatocellular carcinoma	NTP, 1980a
Mouse (female)	gavage	0-0.2 µg/kg bw/week	104 weeks	105-107 weeks	50	Hepatocellular carcinoma, follicular-cell adenomas of the thyroid	NTP, 1980a
Mouse (male)	gavage	0-7.0 µg/kg bw/week	365 days	424-649 days	45	Liver tumors	Toth et al., 1979
Mouse (male)	dermal	0-0.03 µg/week	104 weeks	104 weeks	30	Fibrosarcoma of the integumentary system	NTP, 1980b
Mouse (female)	dermal	00.015 µg/week	104 weeks	104 weeks	30	Fibrosarcoma of the integumentary system	NTP, 1980b

Toth et al. (1979) administered weekly gavage doses of 0.0, 0.007, 0.7 and 7.0 μg/kg bw to groups of 45 male Swiss mice for 1 year. The relatively short duration of exposure limits the sensitivity of this assay for determining the carcinogenic potency of 2,3,7,8-TCDD.

Van Miller et al. (1977a,b) maintained groups of 10 male Sprague-Dawley rats on diets containing 0.0, 0.001, 0.005, 0.05, 0.5, 1.0, 5.0, 50, 500 or 1000 ppb 2,3,7,8-TCDD for 78 weeks. Based on the food consumption of two rats from each group, these dietary levels resulted in doses of 0.0, 0.003, 0.001, 0.01, 0.1, 0.4, 2.0, 24, 240 and 500 μg/kg bw/week, respectively. The small group sizes (10 rats/group) and relatively short exposure times (78 weeks) limit the usefulness of this study in quantitative risk assessment.

Both the NTP (1980a) study and the Kociba et al. (1978a,b) study contain sufficient animals/group and involved sufficiently long dosing schedules to be used for quantitative risk assessment. Kociba et al. (1978a,b) maintained groups of 50 Sprague-Dawley rats on diets providing doses of 0.0, 0.001, 0.01 or 0.1 μg 2,3,7,8-TCDD/kg bw/day for 2 years. The high dose resulted in reduced lifespans for the female rats, reduced body weight gain in both sexes and signs of tissue toxicity. Statistically significant increases in hepatocellular neoplastic nodules were observed in females at doses of 0.1 and 0.01 μg/kg bw/day. At the high-dose, increases were observed in stratified squamous cell carcinomas of the hard palate and/or nasal turbinates in both sexes, in keratinizing squamous cell carcinomas of the lungs in females, in squamous cell carcinomas of the tongue in males and in hepatocellular carcinomas in females.

In the NCI bioassay (NTP, 1980a), groups of 50 Osborne-Mendel rats or Swiss mice were dose twice weekly by gavage with 2,3,7,8-TCDD in a 9:1 corn oil:acetone solution. The rats and male mice received TWA doses of 0.0, 0.0014, 0.0071 and 0.071 μg/kg bw/day. The corresponding doses for female mice were 0.0, 0.0057, 0.029 and 0.29 μg/kg bw/day. Dose-related depressions in mean body weight were reported for both sexes of rats. In male rats, a dose-dependent increase in the incidence of follicular-cell adenomas or carcinomas of the thyroid was observed. The incidence of subcutaneous tissue fibromas was significantly increased in the high-dose group. In female rats, observed increases in the incidence of subcutaneous tissue fibrosarcomas, adrenal cortical adenomas and hepatocellular carcinomas and neoplastic nodules were statistically significant in the high-dose group. In mice, statistically significant increases in tumor incidences were observed only in the high-dose group. An increase in hepatocellular carcinomas and neoplastic nodules was noted in the males. In females, increases were observed in hepatocellular carcinomas and adenomas, fibrosarcoma, histiocytic lymphoma, thyroid follicular-cell adenoma and cortical adenoma or carcinoma.

Quantification of Carcinogenic Risk

A summary of 95% upper-limit human carcinogenic potency estimates for 2,3,7,8-TCDD derived from the Kociba et al. (1978a,b) and NCI (NTP, 1980a) studies in rats and mice, with two pathologists' findings for the Kociba study, are given in Table VIII-5. These potency estimates have been calculated using the linearized multistage model by a previously described methodology (Federal Register, 1980). The largest of these potency factors (q_1^*) comes from data in an independent pathologist's (Dr. R. Squire) review of the Kociba feeding study of female Sprague-Dawley rats. An

TABLE VIII-5

Summary of Human Potency Estimates for 2,3,7,8-TCDD[a]

Species	Study	Sex	Pathologist	Human Potency Estimate q_1^* in $(mg/kg/day)^{-1}$
Rat	Kociba et al.[b]	male	Kociba	1.47×10^4
			Squire	1.73×10^4
Rat	Kociba et al.[b]	female	Kociba	
			Unadjusted	2.52×10^5
			Adjusted for early mortality	1.51×10^5[c]
			Squire	
			Unadjusted	4.25×10^5
			Adjusted for early mortality	1.61×10^5[c]
Rat	NCI[d]	female	NCI-reviewed	3.28×10^4
Mouse	NCI[d]	male	NCI-reviewed	7.52×10^4
Mouse	NCI[d]	female	NCI-reviewed	4.56×10^4

[a] Source: U.S. EPA, 1984b

[b] Kociba et al., 1978a,b

[c] Values used to determine the geometric mean of 1.56×10^5 $(mg/kg/day)^{-1}$

[d] NTP, 1980a

adjustment for high early mortality in the high dose groups led to a slightly lower estimate. The mean of the two pathologists' estimates after mortality adjustment is as follows:

$$q_1^* = [(1.51 \times 10^5) \times (1.61 \times 10^5)]^{1/2} = 1.56 \times 10^5 \text{ (mg/kg/day)}^{-1}$$

These potency estimates were derived from the Kociba feeding study. The responses and parameters of the Kociba feeding study in female rats are given in Table VIII-6. The number with tumors refers to the number of animals with at least one of liver, lung, hard palate or nasal turbinate tumors. Adjustment for early mortality refers to eliminating those which animals died during the first year of study. The first tumor appeared in the high-dose group during the thirteenth month.

With these parameters, the mean 95% upper-limit carcinogenic potency factor for humans, q_1^*, is 1.56×10^5 (mg/kg/day)$^{-1}$. For a 70 kg human drinking 2 ℓ water/day, the water concentration should be $<2.2 \times 10^{-6}$ µg/ℓ in order to keep the upper-limit individual lifetime cancer risk $<10^{-5}$. Water concentration corresponding to excess cancer risk of 10^{-4} and 10^{-6} are, therefore, $<2.2 \times 10^{-5}$ and $<2.2 \times 10^{-7}$, respectively.

Special Considerations

Synergistic Effects.

Enzyme Induction -- 2,3,7,8-TCDD has been demonstrated to significantly alter the toxicity of other toxicants, primarily as a result of enzyme induction (see the Interactive Effects Section in Chapter VI). These changes may either increase toxicity, if metabolism is predominantly an activation pathway, or decrease toxicity if metabolism is predominantly a detoxification mechanism. Thus, Grieg (1972) has demonstrated a 54%

TABLE VIII-6

Responses and Parameters of the Kociba Feeding Study*

Dose (mg/kg/day)	No. with Tumors/No. Examined Adjusted for Early Mortality	
	Squire	Kociba
0	16/85	9/85
0.001×10^{-3}	8/48	3/48
0.01×10^{-3}	27/48	18/48
0.1×10^{-3}	34/40	34/40

le = 720 days
Le = 720 days
L = 720 days

W_h = 70 kg
W_0 = 0.450 kg
R = 5000 ℓ/kg

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*Source: Kociba et al., 1978a,b

decrease in the duration of zoxazolamine-induced paralysis and a 2-fold increase in hexabarbitone sleeping time.

Cocarcinogenesis and Promotion -- In addition to being a complete carcinogen, 2,3,7,8-TCDD has been demonstrated to function as a promoter of DEN-initiated hepatocarcinogenesis (Pitot et al., 1980). Positive results for tumor promoting activity have also been obtained when 2,3,7,8-TCDD was tested on the skin of mice homozygous for the "hairless" trait (Poland et al., 1982). Another attempt to demonstrate the tumor promoting ability of 2,3,7,8-TCDD on mouse skin ("wild" type) have produced negative results (NTP, 1980b; Berry et al., 1978, 1979). 2,3,7,8-TCDD has also been demonstrated to be cocarcinogenic with 3-methylcholanthrene (Kouri et al., 1978).

High Risk Subpopulations. The data from human studies are insufficient to establish the existence of sensitive subpopulations, though there is suggestive evidence that children may be more sensitive than are adults (see the High Risk Subpopulations Section in Chapter VI).

Summary

The recommended HAs developed in this document are summarized in Table VIII-7. The 1-day HA is based on a single-dose LOAEL in the most sensitive species, the guinea pig (Turner and Collins, 1983). The 10-day HA is calculated by dividing the 1-day HA by 10. An AADI for noncarcinogenic effects from lifetime exposure is derived from the LOAEL in the 3-generation reproductive study by Murray et al (1979) along the rationale developed by EPA; however, the carcinogenicity risk assessment based on the linearized multistage model and the carcinogenicity data in the Kociba et al. (1978a,b, 1979) study indicates lower HAs for lifetime exposure.

TABLE VIII-7

Health Advisories for 2,3,7,8-TCDD

	μg/ℓ
1-day (adult)	3.5×10^{-3}
1-day (child)	1.0×10^{-3}
10-day (adult)	3.5×10^{-5}
10-day (child)	1.0×10^{-5}
Lifetime	3.5×10^{-5}
10^{-4} Excess Cancer Risk	2.2×10^{-5}
10^{-5} Excess Cancer Risk	2.2×10^{-6}
10^{-6} Excess Cancer Risk	2.2×10^{-7}

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